CANADIAN THESES ON MICROFICHE

I.S.B.N.

THESES CANADIENNES SUR MICROFICHE

National Library of Canada
Collections Development Branch

Canadian Theses on
Microfiche Service

Ottawa, Canada
K1A 0N4

Bibliothèque nationale du Canada
Direction du développement des collections

Service des thèses canadiennes
sur microfiche

## NOTICE

The quality of this microfiche is heavily dependent upon the quality of the original thesis submitted for microfilming. Every effort has been made to ensure the highest quality of reproduction possible.

If pages are missing, contact the university which granted the degree.

Some pages may have indistinct print especially if the original pages were typed with a poor typewriter ribbon or if the university sent us a poor photocopy.

Previously copyrighted materials (journal articles, published tests, etc.) are not filmed.

Reproduction in full or in part of this film is governed by the Canadian Copyright Act, R.S.C. 1970, c. C-30. Please read the authorization forms which accompany this thesis.

## AVIS

La qualité de cette microfiche dépend grandement de la qualité de la thèse soumise au microfilmage. Nous avons tout fait pour assurer une qualité supérieure de reproduction.

S'il manque des pages, veuillez communiquer avec l'université qui a conféré le grade.

La qualité d'impression de certaines pages peut laisser à désirer, surtout si les pages originales ont été dactylographiées à l'aide d'un ruban usé ou si l'université nous a fait parvenir une photocopie de mauvaise qualité.

Les documents qui font déjà l'objet d'un droit d'auteur (articles de revue, examens publiés, etc.) ne sont pas microfilmés.

La reproduction, même partielle, de ce microfilm est soumise à la Loi canadienne sur le droit d'auteur, SRC 1970, c. C-30. Veuillez prendre connaissance des formules d'autorisation qui accompagnent cette thèse.

THIS DISSERTATION
HAS BEEN MICROFILMED
EXACTLY AS RECEIVED

LA THÈSE A ÉTÉ
MICROFILMÉE TELLE QUE
NOUS L'AVONS REÇUE

NL-339 (r. 82/08)

Canada

31

0-315-15941-3

National Library of Canada | Bibliothèque nationale du Canada

Canadian Theses Division | Division des thèses canadiennes

Ottawa, Canada
K1A 0N4

63850

## PERMISSION TO MICROFILM — AUTORISATION DE MICROFILMER

• Please print or type — Écrire en lettres moulées ou dactylographier

Full Name of Author — Nom complet de l'auteur

AUDIA ZEANE URQUHART

| Date of Birth — Date de naissance | Country of Birth — Lieu de naissance |
|---|---|
| May 11/55 | JAMAICA |

Permanent Address — Résidence fixe

33 NAUTILUS AVENUE
KINGSTON 17
JAMAICA, WI.

Title of Thesis — Titre de la thèse

QUALITY OF MILK PRODUCTS RELATED TO FEEDING PROTECTED LIPID FEED SUPPLEMENTS

University — Université

UNIVERSITY OF ALBERTA

Degree for which thesis was presented — Grade pour lequel cette thèse fut présentée

MSc

| Year this degree conferred — Année d'obtention de ce grade | Name of Supervisor — Nom du directeur de thèse |
|---|---|
| 1983 | DR. P. JELEN |

Permission is hereby granted to the NATIONAL LIBRARY OF CANADA to microfilm this thesis and to lend or sell copies of the film.

The author reserves other publication rights, and neither the thesis nor extensive extracts from it may be printed or otherwise reproduced without the author's written permission.

L'autorisation est, par la présente, accordée à la BIBLIOTHÈQUE NATIONALE DU CANADA de microfilmer cette thèse et de prêter ou de vendre des exemplaires du film.

L'auteur se réserve les autres droits de publication; ni la thèse ni de longs extraits de celle-ci ne doivent être imprimés ou autrement reproduits sans l'autorisation écrite de l'auteur.

| Date | Signature |
|---|---|
| Nov. 26/82 | Audia Urquhart |

NL-91 (4/77)

THE UNIVERSITY OF ALBERTA

# QUALITY OF MILK PRODUCTS RELATED TO PROTECTED LIPID FEED SUPPLEMENTS

by

© Audia Z. Urquhart

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF Master of Science

Department of Food Science

EDMONTON, ALBERTA

Spring 1983

THE UNIVERSITY OF ALBERTA

**RELEASE FORM**

NAME OF AUTHOR

Audia Z. Urquhart

TITLE OF THESIS

QUALITY OF MILK PRODUCTS RELATED TO PROTECTED LIPID FEED SUPPLEMENTS

DEGREE FOR WHICH THESIS WAS PRESENTED Master of Science

YEAR THIS DEGREE GRANTED Spring 1983

Permission is hereby granted to THE UNIVERSITY OF ALBERTA LIBRARY to reproduce single copies of this thesis and to lend or sell such copies for private, scholarly or scientific research purposes only.

The author reserves other publication rights, and neither the thesis nor extensive extracts from it may be printed or otherwise reproduced without the author's written permission.

(SIGNED) Audia Urquhart

PERMANENT ADDRESS:

33 NAUTILUS AVENUE

KINGSTON 17

JAMAICA W.I.

DATED November

1982

THE UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled QUALITY OF MILK PRODUCTS RELATED TO PROTECTED LIPID FEED SUPPLEMENTS submitted by Audia Z. Urquhart in partial fulfilment of the requirements for the degree of Master of Science in Food Processing.

.....

Supervisor

.....

.....

.....

Date Nov. 26/82

## DEDICATION

To My Parents

*Hope ever urges on, and tells us tomorrow*
*will be better*

*-Albius Tibullus.*

## ABSTRACT

Three experiments were designed to evaluate the effect of feeding low doses of canola-based protected lipid feed supplement (PLFS or "Protec") to lactating dairy cows on milk and butter quality. The first experiment included the determination of a threshold level of "Protec" supplementation at which minimum changes in milk and butter quality were noted when 0, 3, 6, and 9% "Protec" were fed to lactating Holstein cows. In the second experiment, susceptibility of "Protec" to oxidation was evaluated and the quality of milk and butter was examined when highly oxidized "Protec" was fed at the 6% level of incorporation. The third experiment entailed the assessment of milk and butter produced from commercial dairy herds which had been fed "Protec" for at least three years.

The inclusion of commercially available "Protec" had no adverse effects on feed intake, milk yield, milk and butter composition and their quality. A possible threshold level of "Protec" supplementation appeared to be about 6% of grain portion of the daily ration. At this level improved spreadability of butter was noticeable in sensory, chemical and physical tests, possibly due to increased polyunsaturated fatty acid content; this could be a conceivable advantage over standard commercial butters. No increase in susceptibility of milks to oxidation occurred with "Protec"-supplemented diets, however, there was a slight decrease in the proneness of these milks to

hydrolytic rancidity, based on the ADV's. The "Protec" became oxidized during storage at a high temperature (40°C), as determined by a substantial increase in its peroxide value. Feeding the oxidized "Protec" produced no off-flavours or oxidative instability of milk and butters obtained. However, the oxidized supplement appeared to negate the positive effects of fresh "Protec", such as increased polyunsaturation and decreased hardness of butter.

The effects of long-term usage of "Protec" in the diets of lactating Holstein cows in commercial farm situations were not as pronounced as noted in experimental trials. Milk fat content was not higher than that obtained without feeding PLFS. Also, there were no significant differences in unsaturation, hardness, and oiling off between butters made from milks produced with and without feeding "Protec". Herd management techniques, stage of lactation of cows, or feed intake patterns may have been possible factors contributing to these less pronounced trends.

It can be concluded that the feeding of canola-based protected lipid at low doses (5-10% of grain portion) should have no detrimental effects on milk and butter quality. However, the improved spreadability of butters produced could be an attractive property for the consumer. Thus, it can be recommended that the current practice of feeding low levels of PLFS as a high energy supplement be continued by those farmers who find it to be economically advantageous.

## ACKNOWLEDGEMENTS

Sincere gratitude and appreciation is extended to Dr. P. Jelen, project supervisor for his trust and guidance throughout my graduate studies.

Many thanks to my committee members Dr. J. Kennelly, Dr. B. Lowrie, and Dr. P. Sporns for their valuable participation.

I am truly grateful to my fellow students and dear friends who provided encouragement, support and laughs when really needed.

Finally, to my aunt and uncle, without whom this would not be possible ....Thank You.

## Table of Contents


| Chapter | Page |
|---|---|
| 1. INTRODUCTION | 1 |
| 1.1 Farming aspects related to milk production | 1 |
| 1.2 Use of PLFS in Alberta | 2 |
| 2. LITERATURE REVIEW | 4 |
| 2.1 Effects of feeding practices on milk production of lactating dairy cows | 4 |
| 2.1.1 Energy considerations | 4 |
| 2.1.2 Fat utilization | 6 |
| 2.1.3 Biochemistry of milk fat synthesis | 8 |
| 2.2 Protected Lipid Feed Supplements | 11 |
| 2.2.1 What are Protected Lipid Feed Supplements | 11 |
| 2.2.2 Manufacture of PLFS | 14 |
| 2.2.3 Protected Lipid Feed Supplement in the diet of dairy cows | 16 |
| 2.3 Effects of protected lipid supplementation | 21 |
| 2.3.1 Intake and efficiency of energy utilization by cattle | 21 |
| 2.3.2 Effect of Protected Lipid Supplement on Milk Composition | 22 |
| 2.3.3 Effect of Protected Lipid Supplement on Oxidative Changes in Milk | 25 |
| 2.3.4 Effect of Protected Lipid Supplement on Butter Characteristics | 27 |
| 2.3.5 Practical implications associated with the use of PLFS | 29 |
| 2.4 Chemistry of fat changes in milk and dairy products | 29 |
| 2.4.1 Milk fat globule membrane | 30 |

2.4.2 Fat oxidation and its measurement .....31

2.4.3 Effect of storage temperature on rate of oxidation .....35

2.4.4 Effect of oxygen level on oxidation .....35

2.4.5 Oxidation as affected by heat treatment ....36

2.4.6 Effect of light exposure on oxidation .....36

2.4.7 Effect of acidity on oxidation .....38

2.4.8 Oxidation as affected by homogenization ....39

2.4.9 Lipolysis .....39

2.4.10 Measurement of lipolysis .....42

3. OBJECTIVES AND EXPERIMENTAL DESIGN .....44

3.1 Experiment 1 - Determination of PLFS Threshold ....44

3.2 Experiment 2 - Storage Stability of "Protec" and Effect of Feeding Stored "Protec" on Milk and Butter Quality .....47

3.3 Experiment 3 - Evaluation of Milk and Butter from Cows being fed "Protec" in Commercial Dairy Herds .....48

4. MATERIALS AND METHODS .....50

4.1 Raw Materials .....50

4.1.1 Protected Lipid Feed Supplement .....50

4.1.2 Microstructure of "Protec" .....50

4.1.3 Formulated Diets .....52

4.1.4 "Stored Protec" .....52

4.2 Processing .....55

4.2.1 Collection of Milk .....55

4.2.2 Homogenization of Milk .....55

4.2.3 Pasteurization of Milk .....56

4.2.4 Separation of Cream .....56

4.2.5 Butter-making .....56

4.2.6 Storage of Milk and Butter .....58

4.3 Susceptibility of Milk to Induced Oxidative and Hydrolytic Rancidity .....58

4.3.1 Copper-induced oxidation of Milk .....58

4.3.2 Exposure of Milk to Fluorescent Light .....59

4.3.3 Inducement of Hydrolytic Rancidity in Milk .....59

4.3.4 Oxidative Stability of Butter .....59

4.4 Proximate Analyses .....59

4.4.1 Diets .....60

4.4.2 Milk .....61

4.4.3 Butter .....61

4.5 Chemical and Physical Analyses of Milkfat .....62

4.5.1 Oxidative Stability by TBA Test .....62

4.5.2 Oxidative Stability by Peroxide Value Determination .....62

4.5.3 Determination of Free Fatty Acid Content ...63

4.5.4 Level of Unsaturation .....64

4.5.5 Hardness .....64

4.5.6 Softening Point .....65

4.5.7 Dropping Point .....66

4.5.8 Oiling Off .....66

4.5.9 Solid Fat content .....67

4.6 Sensory Evaluation of Milk .....67

4.7 Sensory Evaluation of Butter .....69

4.8 Grading of Butter .....70

5. RESULTS AND DISCUSSION .....71

5.1 Experiment I: Effects of feeding graded levels of "Protec" on the quality of milk and butter .....71

5.1.1 Effect of feeding "Protec" on feed consumption, milk yield and milk composition of lactating dairy cows .....71

5.1.2 Effect of "Protec" on the composition of milk and butter used in quality studies ....74

5.1.3 Sensory characteristics of milk .....77

5.1.4 Susceptibility of raw milk to induced hydrolytic rancidity .....79

5.1.5 Susceptibility of milk to induced oxidative changes .....79

5.1.6 Effect of "Protec" on butter characteristics .....82

5.1.6.1 Sensory characteristics of butter .....82

5.1.6.2 Oxidative stability of butters ....85

5.1.6.3 Level of unsaturation of butters .....85

5.1.6.4 Hardness of butter .....87

5.1.6.5 Oiling-off of butter .....90

5.1.6.6 Softening point of butter .....90

5.1.6.7 Dropping point of butter .....91

5.1.6.8 Solid fat content of butters at different temperatures .....92

5.1.7 Discussion .....96

5.2 Experiment II -- Storage stability of "Protec" and the effect of feeding "Stored Protec" on milk and butter quality .....102

5.2.1 Susceptibility of "Protec" to oxidative changes during storage .....102

5.2.2 Effect of "Stored Protec" on feed consumption, milk yield , milk composition and quality of milk and butter .....102

5.2.2.1 Feed Consumption .....104

5.2.2.2 Milk yield and composition .....104

5.2.2.3 Sensory evaluation of milk and butter .....107

5.2.2.4 Susceptibility of raw milk to induced hydrolytic rancidity .....109

5.2.2.5 Susceptibility of homogenized and pasteurized milk to induced oxidative changes .....109

5.2.2.6 Oxidative stability of butter ....113

5.2.2.7 Effect of fresh and "Stored Protec" on butter characteristics .....113

5.2.3 Discussion .....117

5.3 Experiment III-- Evaluation of milk and butter from cows fed "Protec" in commercial dairy herds .....120

5.3.1 Composition of milk and butter .....120

5.3.2 Flavour of milk and butter resulting from "Protec" supplementation .....122

5.3.3 Susceptibility of butter to oxidation .....124

5.3.4 Butter characteristics .....124

5.3.5 Discussion .....127

6. CONCLUSIONS AND RECOMMENDATIONS .....129

6.1 Effect of "Protec" on milk quality .....129

6.2 Recommendations for future research .....130

7. REFERENCES .....132

8. APPENDICES .....150

## List of Tables


| Table | | Page |
|---|---|---|
| 3.1 | Analytical Procedures performed on Milk and Butter | 45 |
| 3.2 | Formulation of Test Concentrate (Experiment 1) | 46 |
| 4.1 | Standard Rolled Ration (Control #1) Fed by University of Alberta Dairy Research Unit | 53 |
| 4.2 | Fat Content of Creams used in Buttermaking | 57 |
| 5.1 | Composition of Concentrate | 72 |
| 5.2 | Effect of "Protec" on Feed Consumption, Milk Yield and Milk Composition of Lactating Dairy Cows | 73 |
| 5.3 | Effect of "Protec" on Yield and Butterfat Levels of Milk from Individual Cows | 75 |
| 5.4 | Effect of "Protec" on the Composition of Pooled Milks and Corresponding Butters | 76 |
| 5.5 | Expt I. Panelists Correctly Identifying Odd Milk Sample in Triangle Tests and Associated Levels of Significance | 78 |
| 5.6 | Susceptibility of milk to oxidative rancidity | 80 |
| 5.7 | Panelists correctly identifying odd butter sample in Triangle Test and associated levels of significance | 83 |
| 5.8 | Effect of "Protec" on butters obtained from milks of individual cows | 84 |
| 5.9 | Inter-relationships among butter characteristics as depicted by Pearson's Correlation Coefficients | 88 |
| 5.10 | Solid Fat Content of Butters | 93-94 |
| 5.11 | Storage stability of "Protec" | 103 |
| 5.12 | Effect of fresh and "Stored Protec" on feed consumption, milk yield and milk composition of lactating dairy cows | 105 |

Table Page

5.13 Effect of fresh and "Stored Protec" on the composition of milk and butter used for quality studies .....106

5.14 Experiment II: Panelists correctly identifying the odd milk sample in triangle tests and the probability levels of significance .....108

5.15 Experiment. II: Panelists correctly identifying odd butter sample in triangle test and probability levels of significance .....110

5.16 Experiment. II: Comparison of butter flavour using Signal Detection .....111

5.17 Experiment. II: Effect of fresh and "Stored Protec" on the susceptibility of milk and butter to hydrolytic and oxidative rancidity .....112

5.18 Effect of fresh and "Stored Protec" on butter characteristics .....114

5.19 Effect of "Protec" on the composition of milk and butter from commercial dairy herds .....121

5.20 Experiment. III: Panelists correctly identifying odd milk sample in triangle test and probability levels of significance .....123

5.21 Experiment. III: Comparison of Butter Flavour Using Signal Detection .....125

5.22 Effect of "Protec" on the Quality of Butter from Commercial Herds .....126

## List of Figures


| Figure | | Page |
|---|---|---|
| 2.1 | Fat metabolism in ruminant | 7 |
| 2.2 | Metabolites used in the synthesis of milk fatty acids | 10 |
| 2.3 | Diagrammatic representation of the digestion of protected lipid feed supplements | 13 |
| 2.4 | Flow Sheet for preparation of oil-seed supplements | 15 |
| 2.5 | Typical changes during storage in the ether extract fraction of a feedingstuff containing unsaturated fat | 19 |
| 2.6 | Some routes of decomposition of fat hydroperoxide | 20 |
| 2.7 | Mechanism of autocatalytic oxidation of lipids | 32 |
| 2.8 | Proposed TBA reaction | 34 |
| 4.1 | Diagram of "Protec" Manufacture | 51 |
| 5.1 | Susceptibility of milk to hydrolytic rancidity | 81 |
| 5.2 | Effect of "Protec" on the level of unsaturation of butters | 86 |
| 5.3 | Effect of "Protec" on butter hardness | 89 |
| 5.4 | Effect of "Protec" on the Solid Fat content of butters | 116 |

# 1. INTRODUCTION

## 1.1 Farming aspects related to milk production

Several factors such as lactation, inheritance, season, environment temperature and feeds are known to have an effect on milk yield and composition (Johnson, 1980). Of these, the feeding regime is the most easily manipulated in order to provide optimum realization of the dairy cow's potential. Well-fed cows (before calving) have been known to produce milk of higher fat content and decreased solids non-fat during the first three months of lactation compared to cows which were poorly fed (Johnson, 1980). Over-feeding, on the other hand, causes an increase in the solids-non-fat. Generally, the fat moiety of the milk is more responsive to changes in the diet of the ruminant than is lactose and protein.

The present milk pricing system operates such that the farmer is paid according to the milk fat yield. This provides a constant incentive for farmers to improve feeding techniques in order to achieve greater milk fat contents. Usually, any decrease in milk fat is associated with increasing concentrate:roughage ratio (Hutton, 1974). Thus, the amount of concentrate offered cannot be increased too much without sacrificing the milk fat content. The need for energy-efficient diets was established as a requirement for increasing monetary returns to the farmers via increased

production.

## 1.2 Use of PLFS in Alberta

The commercial production of protected lipid feed supplements (PLFS) has been established in the USA, Canada, Australia and New Zealand (Storry and Brumby, 1979). In Alberta, over 350 herds are being fed this supplement (Parr, 1982). Protected tallow, using soybean as the source of protein, was used in early commercial trials, however, in recent years the abundant canola-seed has been utilized in formulating the currently available product, "Protec", manufactured in Alberta by Barrhead Alfalfa and Protec Products Ltd., Barrhead, Alberta. The suggested level of supplementation of "Protec" has been limited to low doses (5-10% of grain portion). Increases in fat and polyunsaturated fatty acids of resulting milks have been observed with such levels locally (Grieve, 1980; Wong *et al.*, 1982), and elsewhere. However, concern has been expressed about the susceptibility of this polyunsaturated milkfat to oxidation (Haase, 1977; Goering *et al.*, 1976). The economic returns for the farmer have to be weighed against any possible losses by the processor due to potential defects in milk and milk products obtained. The main purpose of this study was, therefore, to establish whether the canola-based "Protec", currently used in diets of lactating dairy cows, could cause any detrimental effects

on milk and butter quality.

# 2. LITERATURE REVIEW

## 2.1 Effects of feeding practices on milk production of lactating dairy cows

### 2.1.1 Energy considerations

Net energy of lactation used for maintenance, pregnancy and milk production in lactating dairy cows has been found to be higher than for dry, non-pregnant cows (Hutton, 1974). It has been found that the energy supplied in the diet is the limiting component of most dairy rations (Ensminger and Olentine, 1978). Because of the low concentration of energy in a given volume of feed, the ruminant is often physically full before sufficient energy has been consumed in order to permit maximum realization of the animal's potential (Bines, *et al.*1978). This effect is even more pronounced during the post-calving period when poor appetite unfortunately coincides with increased milk yield, the latter due to favourable hormone balance and peak functioning of milk synthesizing apparatus (Storry and Brumby, 1979). Subsequently, in an effort to meet this deficiency (negative energy balance), energy is mobilized from body reserves; however, this is not usually sufficient in the high producing cow. Since it is fairly obvious that energy plays an important role in maximizing the dairy cow's performance, several sources of energy were investigated (Bines *et al.*,

1978).

Results of such studies indicated that if the carbohydrate moiety in the diet was increased (via increased concentrate), the pattern of fermentation in the rumen would be changed so that absorbed energy would be used for weight gain rather than for milk synthesis (Bines, *et al*. 1978; Brown, 1969). Also, the increased intake would not be accompanied by a proportionate increase in milk output, which inevitably had a depressed fat content. Researchers then turned to fat, as this dietary ingredient has a high calorific value in addition to being able to heighten the efficiency of energy use for milk production (Storry, 1981). However, it was soon found that digestibility was depressed when free fat was added to dairy rations. This was attributed to the decrease in fibre digestion resulting from reduced activity of cellulolytic organisms in the rumen (Bines, *et al*. 1978). In addition to this, changes occurred in the amount of methane and volatile fatty acids produced in the rumen which tended to lower the proportion of acetate and butyrate present (Storry,1981). Based on experimental studies, it was suggested that 16% of the total energy available to the dairy cow should be in the form of fatty acids in order for optimum efficiency of energy utilization to occur (Kronfeld, 1976; Brumby *et al*., 1978).

### 2.1.2 Fat utilization

Digestion and absorption of fats in ruminants have been extensively reviewed (Blaxter, 1962; Armstrong and Ross, 1968; Rook and Thomas, 1969; Patton and Jensen, 1976; Christie, 1979; Palmquist and Jenkins, 1980; Storry, 1981). The ruminant digestive tract has three distinct sites of digestion, namely, (a) the reticulo-rumen (rumen) in which food is degraded by microbes, (b) the abomasum and small intestines where digestion is accomplished by enzymes present, and (3), the large intestines (especially the caecum) where a more limited microbial action takes place. It has been established that micro-organisms capable of attacking cellulose, hemi-cellulose, starch, sugar, acid products of carbohydrate digestion, protein, and lipids are present in the rumen, which acts like a large fermentation tank. In general, digesta entering the small intestines contain long chain free fatty acids which are modified by hydrogenation, and to a small extent converted from the cis to the trans form, along with microbial lipids (Fig. 2.1). Bile juices containing phospholipids combine with these products forming stable micelles which are then taken to the microvilli of the small intestines for absorption. Monoglycerides and free fatty acids are absorbed and reformed into triglycerides in the epithelium of the intestine. A fairly high proportion of stearic acid (resulting from hydrogenation of unsaturated fatty acids) enters the duodenum and is subsequently absorbed. The main

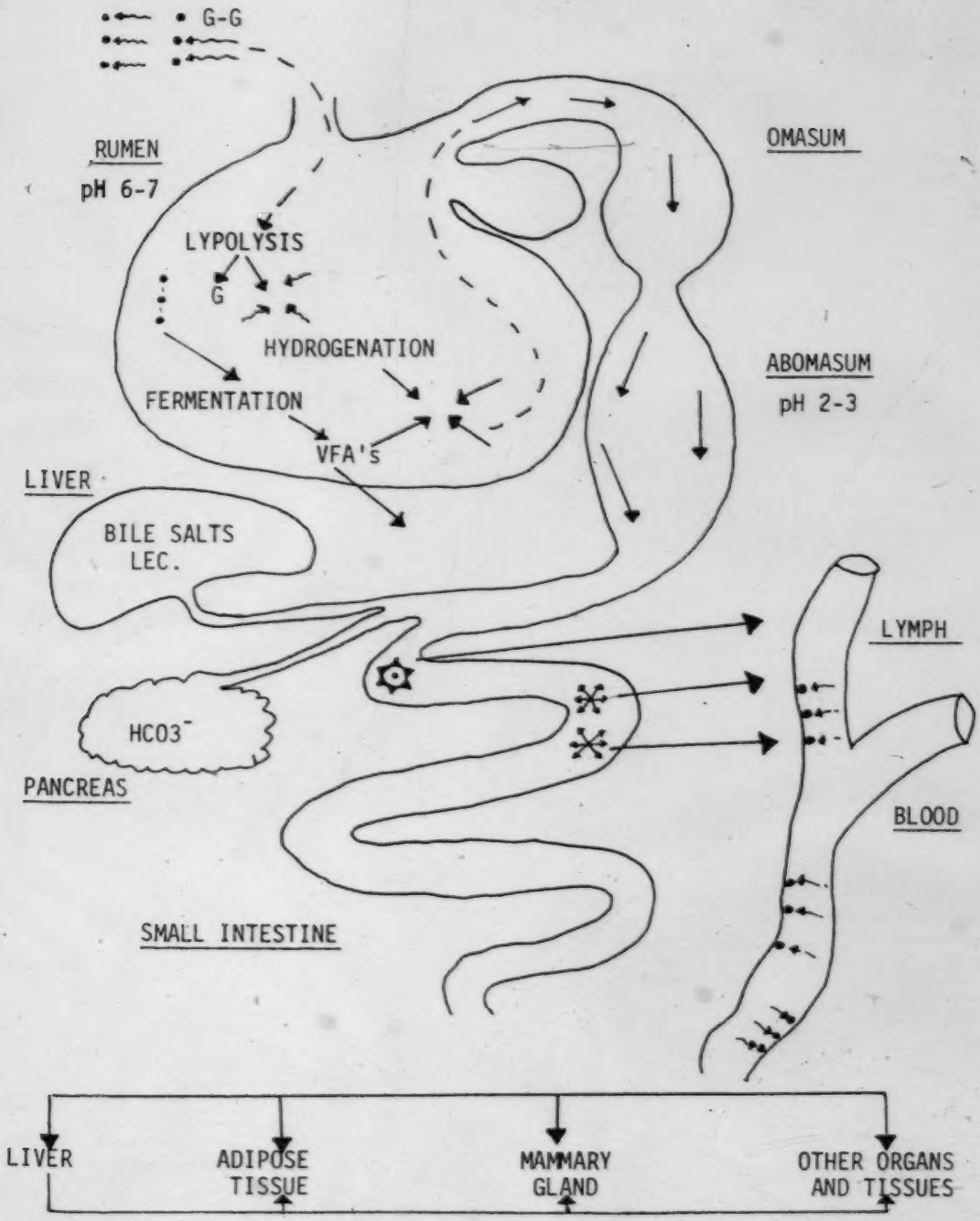



Fig. 2.1 Fat metabolism in ruminant (Adapted from Scott & Cook, 1973)

site of long chain fatty acid (LCFA) absorption is the intestine, where small amounts of fatty acids are absorbed from the upper jejunum (pH 2-4), and the remainder from the lower part of the jejunum (pH 7). Since bacterial lipids do not contain linoleic acid, any requirements for this acid must be supplied to the ruminant through dietary sources that manage to escape hydrogenation. Lipid digestion depends on dietary factors such as the ratio of concentrates to roughage, the amount and type of concentrates and the amount and type of fat in the diet (Storry, 1981).

### 2.1.3 Biochemistry of milk fat synthesis

Milk fat synthesis has been the subject of extensive investigations, warranted probably because the traditional milk pricing system is based on the fat content, or because the amount and type of fat (unlike milk protein and lactose), is subject to changes in response to dietary alterations. The fat in cows' milk occurs almost entirely as triglycerides, the fatty acids being distributed such that one short-chain and two long-chain acids occur for each molecule of glycerol (Brown, 1969). Evidence accumulated so far has established that short chain fatty acids ($C_4$ - $C_{10}$) are synthesized in the mammary gland from acetate and $\beta$-hydroxybutyrate, whereas the $C_{14}$ fatty acids are derived from the $C_{14}$ acids of the blood plasma triglycerides contained in chylomicrons and low density lipoproteins (Patton and Jensen, 1976; Storry, 1972; Storry, 1981). The

medium chain fatty acids may originate from either source as indicated in Fig. 2.2.

The biochemical pathways for milk fat synthesis as understood today can be summarized to include :

i. The malonyl pathway (in cytosol) which involves the carboxylation of acetyl CoA to malonyl CoA. This is thought to be the rate-limiting step and is followed by condensation with acetyl CoA to form fatty acids containing up to 16 carbon atoms (Storry, 1972; Patton & Jensen, 1976).

ii. Direct incorporation of $\beta$-hydroxybutyrate, which maybe subsequently elongated by the addition of acetyl CoA.

iii. Incorporation of acetate into short and intermediate chain fatty acids by mitochondria.

iv. Desaturation of stearic and palmitic acids to corresponding mono-unsaturated acids in microsomes of alveolar cell (Storry, 1981).

In order for the plasma triglyceride fatty acids to be incorporated into milk fat, hydrolysis by lipoprotein lipase has to occur first. The liberated fatty acids and those synthesized in the alveolar cell are then reesterified into triglycerides and finally embodied into the milk fat globule (Storry, 1981). Transfer of dietary fatty acids into milk fat has been noted to be inconsistent. However, since factors such as type of fatty acid provided in the diet, milk fat production, metabolic equilibrium as affected by stage

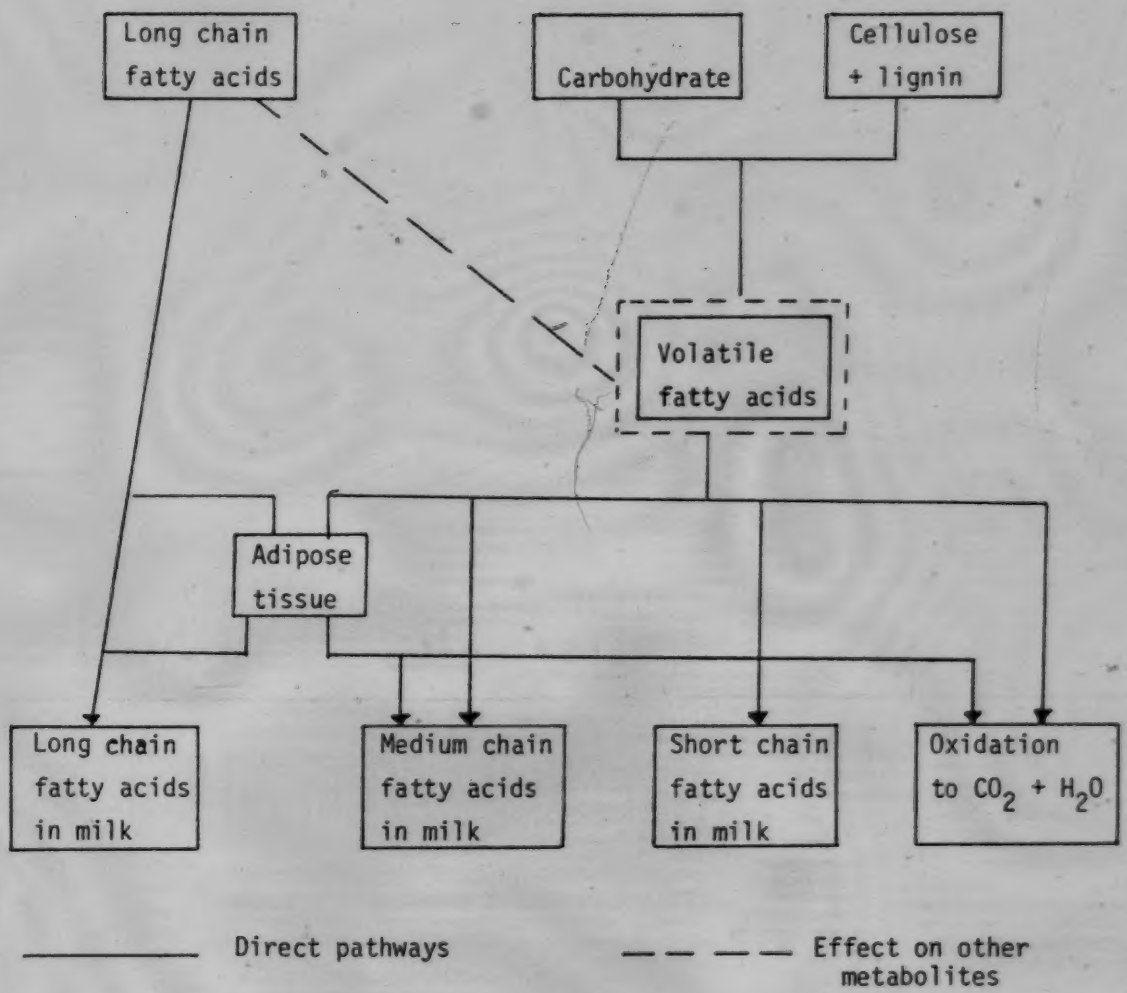



Fig. 2.2 Metabolites used in the synthesis of milk fatty acids

(Adapted from Storry, 1981)

of lactation, and input of glucogenic nutrients influence the % transfer, the potential for variation can be appreciable (Jack and Smith 1956; Palmquist & Mattos, 1978; Smith *et al*.1978; Storry, 1981). With respect to the type of fatty acid provided in the diet, it is worth looking at the occurrence of low milk fat syndrome, which has been reported to occur when large quantities of polyunsaturated oils are fed. It has been ascertained that this phenomenon is due to reduced availability of acetate and decreased mobilization of adipose long chain fatty acid (LCFA) for milk synthesis (Palmquist and Jenkins, 1980). If the polyunsaturated fatty acids fed are in a protected form, the risk of low milk fat syndrome is lowered, while the cow will benefit from the increased energy supplied.

## 2.2 Protected Lipid Feed Supplements

### 2.2.1 What are Protected Lipid Feed Supplements

A significant breakthrough by T.W. Scott and co-workers took place in the early 1970's allowing large quantities of energy-efficient oils and fats to be consumed by ruminants without adverse effects on their metabolism. This was achieved by encapsulating the lipid in a protein envelope which is subsequently treated with formaldehyde. Essentially, the encapsulated fat escapes microbial attack

in the rumen, but becomes fully available for efficient digestion and absorption in the small intestines as illustrated in Fig. 2.3 (Scott *et al.*, 1972; Scott and Cook, 1973; Storry and Brumby, 1979). The encased fats are generally referred to as "protected lipids", and have been used world-wide in both experimental and farm conditions to increase the level of polyunsaturated fats in milk and meat (Plowman *et al.*, 1972; Scott, 1975; Haase,1977; Rook, 1977; Storry and Brumby, 1979). In the majority of studies carried out, the level of incorporation of these protected lipids ranged between 20-30% of concentrate, and the consequences of such levels included (a) increased energy intake of cows, (b) increased milk fat secretion and (c) increase in unsaturation of the milk fat. This approach was prompted by the fact that the intake of polyunsaturated fat was being encouraged for consumers with a high risk for developing coronary heart disease (Haase, 1977; Johnson, 1974).

The realization of the wide practical implication of protected lipid feed supplements in ruminant nutrition provided the necessary impetus for the commercial production of these supplements. Since it was found that the particular fatty acid composition of the protected lipid was reflected in the fatty acid composition of the milk and meat, several oilseeds of varying levels of unsaturation were investigated (Barbano and Sherbon, 1980).

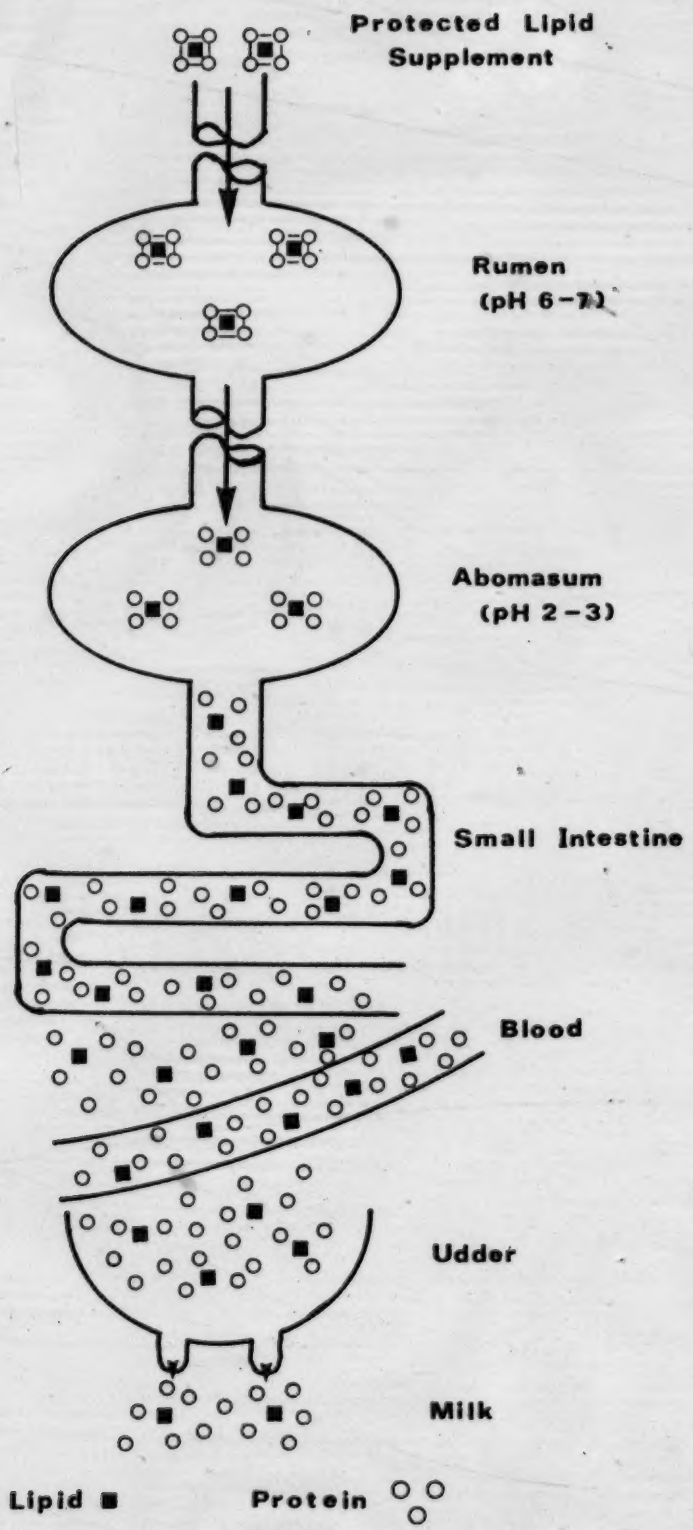



Fig. 2.3 Diagrammatic representation of the digestion of Protected Lipid Feed Supplements

### 2.2.2 Manufacture of PLFS

In the initial production of protected lipid feed supplements (PLFS), encapsulation was achieved by coating finely dispersed oil droplets with a formaldehyde treated protein (usually casein). Vegetable oils obtained from sunflower, safflower, linseed, cottonseed, peanut and coconut have been used in addition to tallow as sources of lipid material in various experimental trials. The spray dried supplement was usually prepared by homogenizing a vegetable oil with a solution of sodium caseinate, and treating with formalin prior to spray drying (Scott, *et al.*, 1971). Although the supplements prepared in this way were satisfactory for experimental use, they proved too expensive for commercial application (Scott, 1975). Scott and Cook (1973) therefore modified the procedure to include natural oilseeds which could supply both the oil and protein needed, hence reducing cost of production. As illustrated in Fig. 2.4, a small amount of additional protein is mixed with the oil in order to provide efficient emulsification and subsequent protection of the polyunsaturated oil. The finely conminuted mixture is treated with sodium hydroxide which is needed to obtain an adequate solution of the seed proteins (Scott *et al.*, 1972). The addition of formalin and polyvalent ions such as $Fe^{3+}$ or $Ca^{2+}$ cause protein-protein interactions - thus trapping the oil. The final product may be in the form of spray dried particles or a gel which can then be ground.

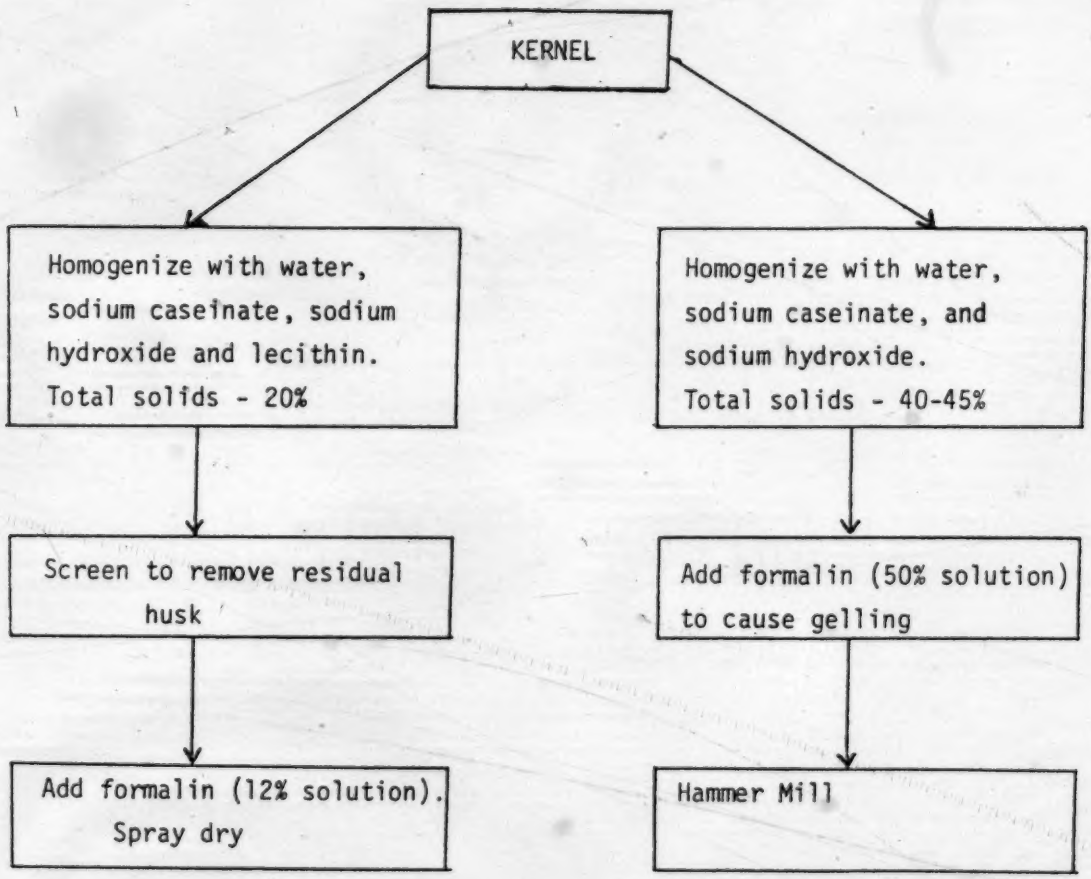

KERNEL
Homogenize with water, sodium caseinate, sodium hydroxide and lecithin. Total solids - 20%
Homogenize with water, sodium caseinate, and sodium hydroxide. Total solids - 40-45%
Screen to remove residual husk
Add formalin (50% solution) to cause gelling
Add formalin (12% solution). Spray dry
Hammer Mill

Fig. 2.4 Flow sheet for preparation of oil-seed supplements
(Adapted from Scott et. al., 1972)

The advantages and disadvantages of using the different oilseeds had to be evaluated when deciding on the appropriate supplement. For example, it was discovered that although safflower has the advantage of a higher oil content and a higher proportion of $C_{18:2}$, its tough husks make emulsification difficult, hence sunflower seeds, which have softer, more easily removed husks are preferred (Scott, 1975; McDonald and Scott, 1977). Presently, sunflower seeds and soybeans are used in Australia, New Zealand, and the USA. Peanuts were tried, but because of the possibility of aflatoxin content, were rejected for this purpose. It has been suggested that canola seed would be potentially useful in Canada due to its availability and triglyceride composition (Haase, 1977; McDonald and Scott, 1977). Canola-based PLFS is now being produced on a commercial scale as per the schematic diagram shown in Fig. 4.1.

**2.2.3 Protected Lipid Feed Supplement in the diet of dairy cows**

The level of supplementation of PLFS in initial studies was tailored in order to produce significant increases in butterfat yield and level of unsaturation of the milk product. For these reasons, researchers such as Scott *et al.* 1970; Plowman *et al.*, 1972; McLeod *et al.*, 1977; Smith *et al.*, 1978, to name a few, used rations of 1-5 kg PLFS/cow/day. With the use of high levels of protected lipid (average 1.7 kg per cow per day), results of Canadian trials

revealed that although milk yield and fat content increased substantially, the productive response was not sufficient to prove definite economic feasibility (Parr, 1980). Palatability and intake problems, as well as fat-cow associated disturbances developed, therefore, lower levels of PLFS inclusion were investigated. Collaborative results of studies carried out by Bines *et al.*, (1978) and Brumby *et al.*, (1978), established that at high levels of protected tallow supplementation (eg. 15% of ruminant's diet), cows received excessive fat energy. This decreased the efficiency of utilizing both protein and carbohydrate, hence they concluded that 5-10% levels of PLFS incorporation would result in the greatest efficiency of utilization of carbohydrate, protein, and fat for milk production. As a consequence of these and other commercial studies, the use of PLFS in Canada is presently limited to 5 - 7.5% of concentrate offered to cows.

Worth mentioning is the fact that most of the diets formulated to include PLFS were isonitrogenous, while some studies were carried out using isocaloric diets. Isonitrogenous diets containing PLFS usually varied less than 10% from standard rations (Bitman *et al.*, 1973; Plowman *et al.*, 1972; Smith *et al.* 1978).

The use of formaldehyde in the manufacture of PLFS was not found to be toxic to the ruminant. It is degraded to methane and carbon dioxide in the rumen (Mills *et al.*, 1972), hence there is no risk of carry-over in the milk.

In formulating diets, it was discovered that pelleting should be discouraged for supplements, as damage to the protein jackets of the particle may occur, resulting in loss of protection (Scott, 1975). In these cases, the common advantages of PLFS usage would occur but only to a lesser extent. With sufficient loss of protection, free oil would probably result and be metabolized in the same way as if unprotected fat was fed. Hence hydrogenation of unsaturated fatty acids, impaired digestion and ultimately low milk fat yield would occur. To avoid such complications, PLFS is usually incorporated in the milled ration with thorough mixing.

Another area of concern is the potential of oxidation to occur in stored feeds containing protected lipid. According to Carpenter (1968), diets containing highly oxidized free oils (as indicated by increased peroxide values) had no detrimental effects on animals' intake, metabolism or performance. He further described the increase and subsequent decrease in peroxide value that occurred during storage of the feed (Fig. 2.5). It was postulated that this pattern was due to the known instability of hydroperoxides formed (Fig. 2.6).

To date, there is a lack of documented experimentation on the potential development of oxidation in the lipid moiety of PLFS, and the effect that this would impart to feeds at particular levels of supplementation. The increased polyunsaturated fat content in protected lipids, at high

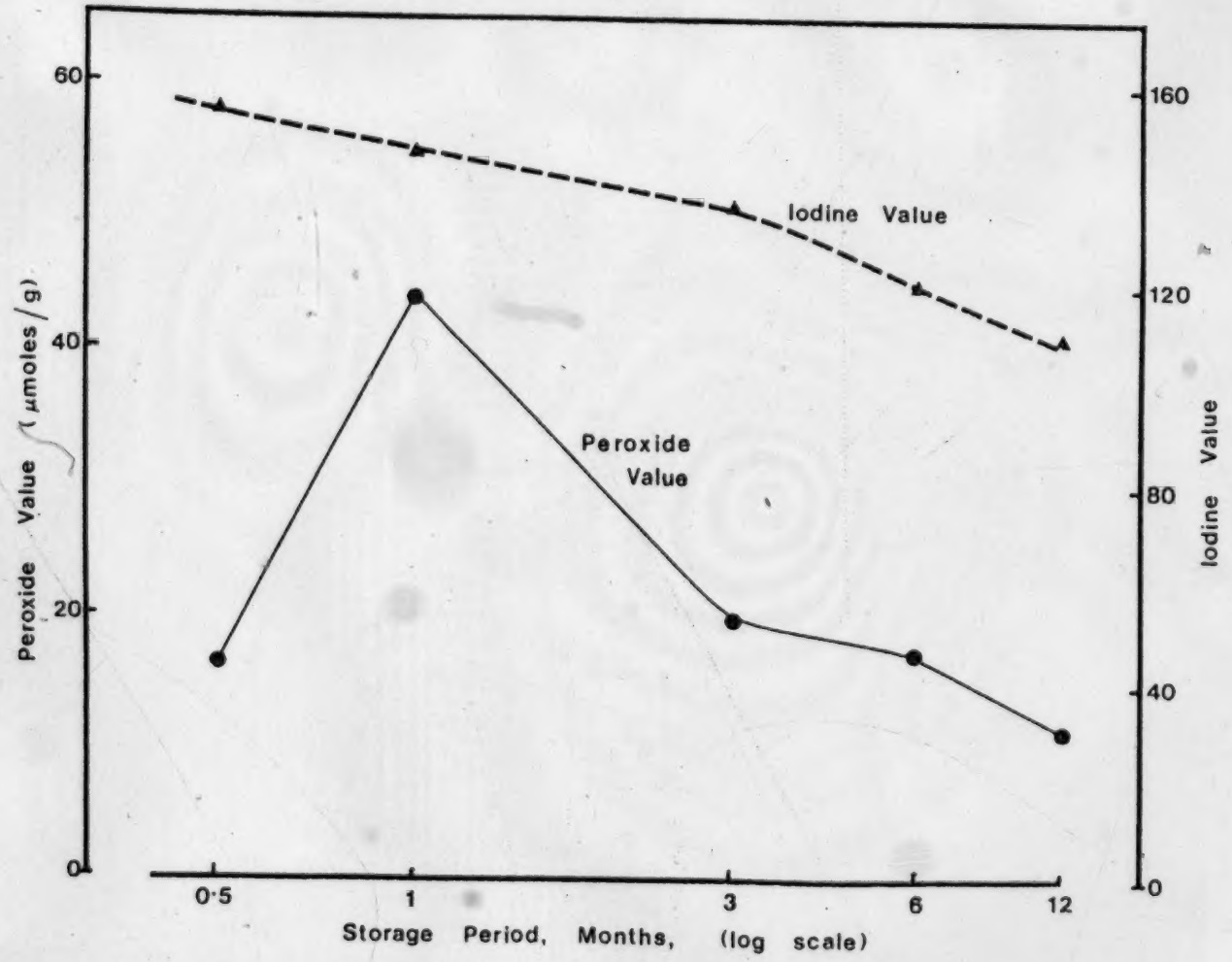



Fig. 2.5 Typical changes during storage in the ether extract fraction of a feedingstuff containing unsaturated fat. (Adapted from Carpenter, 1968)

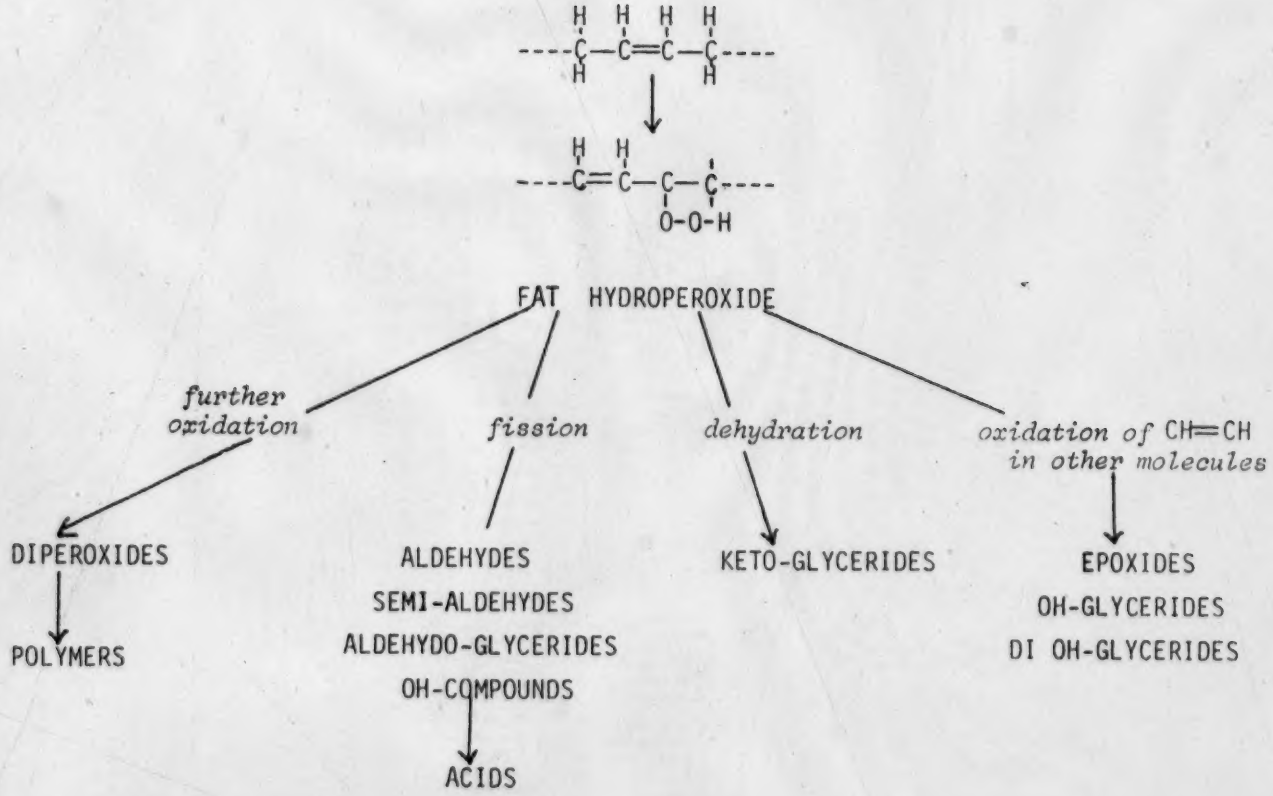



Fig. 2.6 Some routes of decomposition of fat hydroperoxide
(Adapted from Carpenter, 1968)

levels of incorporation, may cause decreased palatability or adverse effects on the ruminant's metabolism if oxidation should occur. Two areas which warrant more attention are:

i. Effect of "protection" on the rate of lipid oxidation if PLFS was stored under adverse conditions

ii. Level of supplementation of abused PLFS needed for adverse effects on ruminant's metabolism to occur.

## 2.3 Effects of protected lipid supplementation

### 2.3.1 Intake and efficiency of energy utilization by cattle

Generally, it has been demonstrated that with cows not fed *ad libitum*, replacement of part of the basal concentrate with PLFS on a weight to weight basis resulted in increased energy intake (Goering *et al.*, 1976; Wrenn *et al.*, 1978; Kronfeld *et al.*, 1980). With *ad libitum* feeding however, dry matter intake decreased, resulting in either no change or an increase in energy intake when low levels of PLFS are fed; while high levels of supplementation caused decreased intakes (McLeod *et al.*, 1977; Bines *et al.*, 1978; Palmquist and Jenkins, 1980). In a very comprehensive review by Storry (1981), several possible explanations were offered for the lack of a distinct effect of PLFS on energy intake. Firstly, it was felt that the different energy requirements and capacities to respond would affect the energy intake of dairy cows in various stages of lactation. Also, the

variation in dietary conditions (especially with respect to type and amount of roughage fed) between experiments could contribute to a change in energy intake. The semi-protected nature of some lipid supplements is also a possible reason, since the presence of free fat that may result would affect dry matter and energy intakes. Finally, it has been suggested that some metabolic or endocrine factor may limit energy intake and dictate negative balance during early lactation.

With respect to energy utilization, it has been found that more efficient use of metabolizable energy for milk production and liveweight gain occurred when protected lipids were fed (Bines *et al.*, 1978; Brumby *et al.*, 1978; Smith *et al.*, 1978; Kronfeld *et al.*, 1980). It was theorized that this effect was due to the increased energy density of these diets, and the more efficient metabolism of LCFA compared to the volatile fatty acids which are the major source of digestible energy in ruminants (Blaxter, 1967; Kronfeld, 1976).

### 2.3.2 Effect of Protected Lipid Supplement on Milk Composition

Data from feeding trials, in which fairly high levels of PLFS supplementation (1.5 - 5.0 kg/cow/day) were used, indicated that almost always the milk yield remained unchanged (Goering *et al.*, 1972 ; Goering *et al.*, 1976; Plowman *et al.*, 1972; Bitman *et al.*, 1973; Smith *et al.*,

1978; Yang *et al.*, 1978; Wong *et al.*, 1982). In some studies, an increase in milk yield resulted (Kristensen *et al.*, 1974). The most noticeable change in milk composition occurs in the milk fat content. Significant increases in the butterfat content have been documented for protected lipids in which different oils and oilseeds have been used at various levels of supplementation. The suppression of intramammary fatty acid synthesis normally associated with the feeding of unprotected oil is minimal when protected oils are used - hence this is a possible explanation for the increase in fat content associated with its use (Storry, 1981). In studies undertaken by Grieve (1976 ; 1980) and Wong *et al.*, (1982) a slight increase in the butterfat content of milk occurred when using low levels (7.5%) of protected tallow in which soybean meal was the source of protein. Other investigators such as Pan *et al.* (1972); Plowman *et al.* (1972); Goering *et al.* (1972); Yang *et al.* (1978); recorded an increase in butterfat content of up to one percentage unit when much higher levels of supplementation were fed.

The nature of the butterfat is such that it reflects the fatty acid composition of the protected lipid. Thus the level of polyunsaturation inevitably increases in milkfat, the extent of which is determined by factors such as the level of supplementation, the fatty acid profile of the oil used, and the stage of lactation. Scott and Cook, (1970); Pan *et al.* (1972); Plowman *et al.* (1972); Scott *et al.*

(1972); Bitman *et al.* (1973); Chandler *et al.* (1973), reported linoleic acid content of up to 35% using safflower oil. Elevated levels of $C_{18:2}$ had little effect on the stereospecific distribution of other major fatty acids when PLFS were fed (Mills *et al.*, 1976; Barbano and Sherbon, 1980). Of interest is the observation that short and medium chain fatty acids (except butyric) were positively related to the carbohydrate and cellulose intake and negatively related to fatty acid intake , whereas yields of $C_{4}$, $C_{14}$, and $C_{16}$ fatty acids were positively related to cellulose and carbohydrate intake, while curvilinear to fatty acid intake (Storry, 1981).

Another advantage of using protected lipids lies in the fact that they can be used to rectify incidence of low milk fat syndrome normally associated with the feeding of low roughage, high concentrate diets (Storry *et al.*, 1974). It was postulated that this was a result of sufficient fatty acid being digested in order to meet the increased demands of the adipose tissue and also in order to compensate for the diminished intramammary synthesis of fatty acids.

The changes in fatty acid composition of milk obtained with protected lipid feeding may have a considerable effect on the measurement of fat and lactose by infra-red methods. This is mainly due to differences in the average molecular weight of component acids (Franke *et al.*, 1977).

With respect to other milk components, variable results have been recorded when protected lipids were fed. A

decrease in the lactose content of milk has been noted by Cook *et al.*, 1972; and Pan *et al.*, 1972, whereas no significant change was recorded by Grieve, 1980, and Storry *et al.* (1978a&b). With respect to protein content, the trend appeared to be a very slight increase at low levels of supplementation, and a slight decrease or no change when higher than 0.5 kg PLFS/day were fed (Storry and Brumby, 1979). Pan *et al.* (1972) and Bitman *et al.* (1973), recorded an increase in protein levels when about 1kg PLFS per day was fed to dairy cows. On the the other hand, results of studies by Mattos and Palmquist (1974), and Grieve (1976), indicated that protein content decreased, while no change in the protein content was reported by Goering *et al.* (1977), and by Barbano and Sherbon (1980). According to Pan *et al.* (1972) and Smith *et al.* (1978), there was a decrease in the solids-non-fat content of milk when PLFS were fed. However no significant change was noted in this parameter in studies carried by Bitman *et al.* (1973), Grieve (1976), and Barbano and Sherbon (1980).

### 2.3.3 Effect of Protected Lipid Supplement on Oxidative Changes in Milk

It is now known that milk in general can be susceptible to deterioration due to oxidative changes ocurring in the milk fat portion (See Section 2.4.2). An increase in the oxidative instability of milk fat has been shown to be related to increases in the linoleic acid content (Smith

*et al.*, 1963; Sidhu *et al.*, 1973). Therefore, since the concentration of this fatty acid is higher in milk resulting from cows being fed PLFS, it is expected that this milk would be sensitive to oxidative deterioration. However, it was pointed out that this adverse effect was operative when protected oils *per se* were used, and that oxidative stability was improved when protected oilseeds were used instead (Haase, 1977). Results of studies carried out using both low and medium levels of incorporation of protected safflower oil revealed that an oxidized flavour developed readily in raw milk and gradually during storage of homogenized and pasteurized milk. Goering *et al.* (1976), who fed 0.8 kg protected safflower oil per day, to lactating Holsteins, suggested that the use of tocopherol would be effective in preventing off-flavours. This was in agreement with results and recommendations by Edmondson *et al.* (1974).

The compensatory decrease in $C_4$ fatty acid that occurs with increase in $C_{18:1}$ (Gooden and Lascelles, 1973; Astrup *et al.*, 1976; Astrup *et al.*, 1979), may cause a reduction in rancid flavour in milk, since this fatty acid ($C_4$) is implicated in the development of rancid flavours. When 0.36 kg/day protected oil was fed, Astrup *et al.*, (1979), reported that in addition to an increase in the oxidized flavour, there was a decrease in the potential for rancid flavour development, as detected by a sensory panel and corresponding free fatty acid contents. Further studies using protected rapeseed oil indicated that such supplements

inhibit the production of rancid flavours (Astrup *et al.*, 1980).

In general, literature data on the relationship between hydrolytic rancidity and PLFS usage appear scant.

### 2.3.4 Effect of Protected Lipid Supplement on Butter Characteristics

The relatively high level of linoleic acid that occurs in milk fat when protected oil supplements are fed to lactating dairy cows inevitably has an effect on flavour, acceptability and shelf life of butter and other milk products (Rook, 1977). In particular, butter has been shown to have an improved spreadability at refrigeration temperatures (Buchanan *et al.*, 1970; Buchanan and Rogers, 1973; Edmondson *et al.*, 1974). It is speculated that this consequence would be favourable to most consumers. Due to the increased softness of such butter, modified churning methods were designed (Kieseker *et al.*, 1974), and it was recommended that temperature control should be practised so that butter can be given enough working in order to incorporate moisture satisfactorily. Tendency to slump and oil off is more pronounced in these "polyunsaturated butter" (Kieseker and Eustace, 1975), thus making them slightly inferior to the luxury margarines (Rook, 1977). This of course is due to the increased proportion of liquid fat. According to Taylor and Norris (1977), the consistency of butter is determined primarily by the proportion of solid

fat, hence more slumping is expected in butter with high polyunsaturated fatty acid content. Improved spreadability of butter, with the concurrent decrease in oiling off was possible when blends of "conventional butter" and "polyunsaturated butter" were made (Wood *et al.*, 1975). Butter containing high linoleic acid contents (approx. 20%) were found to be more susceptible to oxidation on storage, especially if exposed to light or contaminated with copper (Buchanan and Rogers, 1973). However, butter had no adverse flavours after two months refrigerated storage. Manufacture of cultured butter by Kreula and Norlund (1974), containing only 6.1% linoleic acid, displayed improved spreadability, no undesirable flavours, and no oxidative problems on storage at a range of refrigeration temperatures. A ration containing 0.5 kg protected safflower oil was used in this study, thus it is likely that at low levels of supplementation, the small increase in linoleic acid that occurs does not adversely affect the quality of butter produced. However, in an effort to prevent oxidation occurring especially with high levels of supplementation, precautions should be taken to avoid copper contamination; also, the addition of antioxidants has been suggested by some workers in this field (Buchanan *et al.*, 1970; Buchanan and Rogers, 1973; Kieseker *et al.*, 1974; Rook, 1977).

### 2.3.5 Practical implications associated with the use of PLFS

The main area of application for protected lipid feed supplements is in the feeding of high yielding dairy cows in early lactation. In this way one can improve the efficiency of energy use, allow for greater utilization of genetic potential, and specifically increase milk fat and its unsaturation, thus alleviating the low milk fat syndrome (Storry, 1981). However, with the world wide use of protected lipids, an accurate, faster and more routine method of determining the degree of protection of commercial supplements is urgently needed in order to facilitate efficient production (Storry, 1981).

## 2.4 Chemistry of fat changes in milk and dairy products

Cows' milk is simultaneously a solution of low molecular weight compounds, a colloidal dispersion of protein micelles, and an oil-in-water emulsion. At the time of secretion, milk fat exists as an emulsion of microscopic, immiscible liquid fat droplets in the aqueous phase of milk plasma (Brunner, 1980). The stability of this emulsion is a result of the presence of a third phase, a film containing protein and phospholipids on the surface of the dispersed particles. Due to the forces inherent in this microenvironment, the fat particles occur as finely divided spheres individually known as the "milk fat globule". Most of the milk lipids are located in these fat globules, and

their surrounding membrane, while small amounts are also found in the milk serum (Jenness and Patton, 1959). Although the synthesis of milk fat in the mammary gland is not fully understood, it is now known that the long chain fatty acids presumably come from triglycerides absorbed from the blood, while the short chain fatty acids are thought to be synthesized from acetate and $\beta$-hydroxybutyrate (Luick, 1961). On the average, milk fat globules are about 4$\mu$ in diameter, however, they have known to vary in size and distribution depending on environmental influences such as feed, temperature, and condition, ranging from 0.1 to 22.2$\mu$ (Brunner, 1980).

### 2.4.1 Milk fat globule membrane

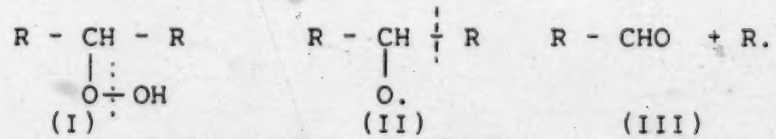
The very existence of the milk fat globules depends on the adsorbed membrane, since without it, coalescence into a continuous fat layer would occur. It is now accepted that the milk fat globule membrane (FGM) consists of protein, phospholipids, cerebrosides, cholesterol, neutral glycerides, and water (Mulder and Walstra, 1974). The lipids in the membrane - especially the cephalin fraction of the phospholipids - contain a large proportion of polyunsaturated fatty acid residues. Therefore, the membrane is susceptible to oxidation (Koops, 1969). The presence of these oxidizable phospholipids together with the large surface area of the globules, sets the scene for oxidation to occur. Since phospholipids are also present in the skim

milk phase, there are three potential sites for lipid oxidation: the fat globules, the material oriented at the surface of these globules, and the aqueous phase (Patton, 1962).

**2.4.2 Fat oxidation and its measurement**

Auto-oxidation of milk lipids is similar to that occurring in other edible oil products. However, the physical state of milk, presence of natural anti- or pro-oxidants, as well as processing and storage conditions tend to influence the rate at which it occurs (Parks, 1980).

Oxidative deterioration of milk proceeds through a free radical chain mechanism involving initiation, propagation, and termination, (Fig. 2.7). This auto-oxidation which often leads to defects in flavour of milk products, occurs mainly in polyunsaturated fatty acid residues which are particularly sensitive to initiation. Hydroperoxides are important products of reaction of fatty acids with oxygen, since they undergo subsequent reactions that terminate in carbonyl compounds responsible for off-flavours (Fig. 2.7). The mechanism that has been proposed by Frankel *et al.* (1961) involves cleavage of the isomeric hydroperoxide (I), to the alkoxyl radical (II), which undergoes carbon-carbon fission to form the aldehyde (III).



# HYDROPEROXIDE THEORY OF LIPID OXIDATION

**A. INITIATION**

$$RH + O_2 \longrightarrow ROOH$$

$$ROOH \longrightarrow \text{FREE RADICALS (e.g. } R^{\bullet}; RO^{\bullet}; RO_2^{\bullet}, \text{ etc.)}$$

**B. PROPAGATION**

$$R^{\bullet} + O_2 \longrightarrow RO_2^{\bullet}$$

$$RO_2^{\bullet} + RH \longrightarrow R^{\bullet} + ROOH$$

**C. TERMINATION**

$$\left.\begin{array}{l} R^{\bullet} + R^{\bullet} \\ R^{\bullet} + RO_2^{\bullet} \\ RO_2^{\bullet} + RO_2^{\bullet} \end{array}\right\} \text{STABLE (NON-RADICAL) END PRODUCTS}$$

Fig. 2.7 **Mechanism of autocatalytic oxidation of lipids.**

Fat oxidation occurring in milk systems can be effectively monitored by the Thiobarbituric Acid Test (TBA), where, malonaldehyde reacts with two molecules of TBA to produce a complex with a characteristic colour (Fig. 2.8).

Since the primary products of lipid oxidation are hydroperoxides, the determination of these compounds as the Peroxide Value has been used as an indication of oxidative state. However, due to the instability of hydroperoxides (Fig. 2.6), the determination of the Peroxide Value is very sensitive to light, oxygen, and temperature changes. The two main sources of error in this measurement are (a) absorption of iodine at unsaturated bonds of the fat, and (b) the liberation of iodine from potassium iodide by dissolved oxygen in the solution to be titrated (Gray, 1978). The official method (AOCS) for determining the Peroxide Value of fats is highly empirical and thus any variation in the procedure may cause inconsistencies in results. Also, it has been documented that it is difficult to determine low Peroxide Values with this test, due to problems with end-point determination (Gray, 1978).

Milk can vary in its susceptibility to oxidized flavour, and it has been reported that some milks may be considered to be spontaneous with respect to oxidation (Bruhn *et al.*, 1976), although the exact mechanism is not well defined. Contamination of milk with copper or iron has been implicated in accelerating the oxidation process (Smith and Dunkley, 1962; Shipe *et al.*, 1978). Natural copper in

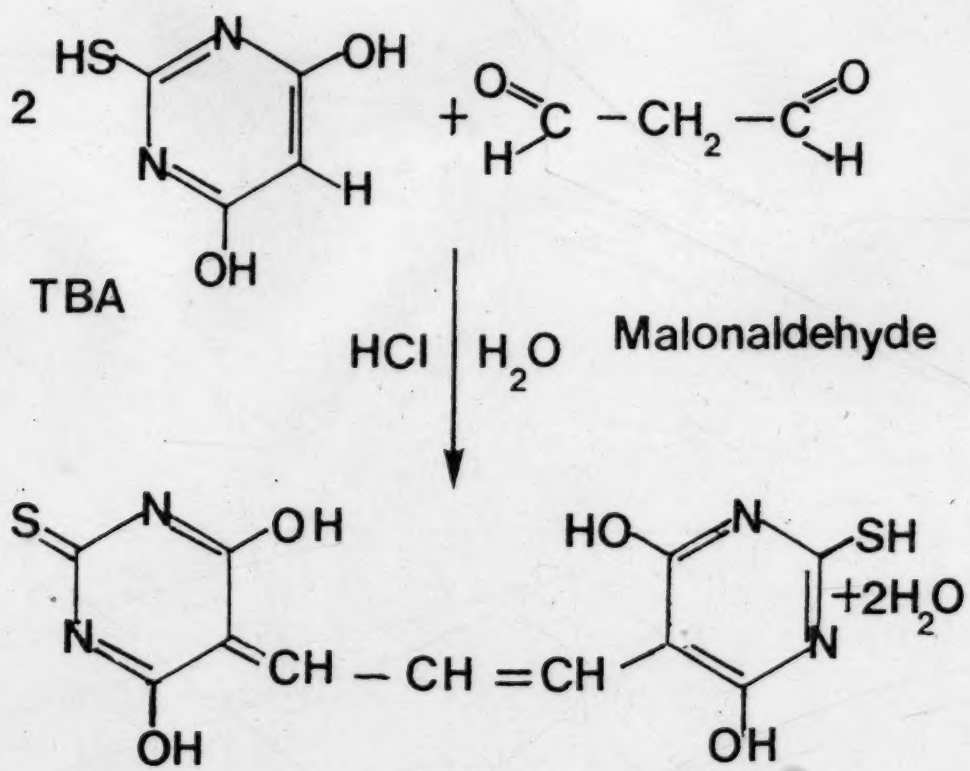



Fig. 2.8 Proposed TBA Reaction
(Sunnhuber et al 1958)

the fat globule membrane amounts to approx. 10 µg/100g. At low storage temperatures, it has been suggested that this copper migrates to the plasma, whereas heating causes migration of added copper from the plasma to the fat globule membrane, where oxidation then occurs (Mulder and Walstra, 1974). In addition to this, it has been found that milk obtained from cows on dry lot feeding is more susceptible to oxidation than that of cows on pasture (Shipe, 1964).

Other factors affecting oxidative deterioration in milk and milk products include oxygen levels, heat treatment, exposure to light, acidity and homogenization.

### 2.4.3 Effect of storage temperature on rate of oxidation

The role of storage temperature is anomalous since it was reported that the intensity of off-flavour and TBA values decreased with increasing storage temperature (Dunkley and Franke, 1967). On the other hand, low storage temperatures tended to decrease the rate of light-induced oxidation in studies carried out by Dunkley *et al.* (1962).

### 2.4.4 Effect of oxygen level on oxidation

It is now understood that a variety of oxygen species (including the hydroxyl radical, singlet oxygen, and ozone) can be generated in or near food systems such as milk, and can ultimately yield peroxides which subsequently decompose to initiate oxidative chain reactions (Korycka-Dahl and Richardson, 1980). Similarly, it has been shown that in

fluid milk, the absence or removal of dissolved oxygen decreases off-flavour development. Singleton *et al.* (1963), demonstrated that oxygen was necessary for the development of light-induced off-flavours, while Sharp (1941), demonstrated that deaeration inhibited off-flavours even in the presence of 0.1 mg Cu/litre of milk.

**2.4.5 Oxidation as affected by heat treatment**

It has been consistently reported that temperatures up to that of pasteurization tend to increase the susceptibility of milk to spontaneous copper-induced and light-induced oxidation (Bergman *et al.*, 1962; Smith and Dunkley, 1962; Parry *et al.*, 1966; Parks, 1980). An inhibitory effect on oxidative deterioration was noted when fluid milk was heated to higher temperatures than that of pasteurization (Greenbank, 1940; Tamsma *et al.*, 1962). It was thought that most heated products do not become oxidized until sulphydryls are first oxidized and the cooked flavour has disappeared (Josephson and Doan, 1939).

**2.4.6 Effect of light exposure on oxidation**

Extensive accounts on the effect of exposure of milk to radiant energy have revealed that off-flavours invariably developed (Aurand *et al.*, 1964; Dunkley and Franke, 1967; Dimick, 1973; Hansen *et al.*, 1975; Shipe *et al.*, 1978). These off-flavours have generally been categorized as being either "activated" or "oxidized".

Activated or sunlight-flavour has been hypothesized to develop rapidly, and has been attributed to the dissociation of a loosely bound complex of riboflavin and tryptophan-containing milk protein (Aurand *et al.*, 1964). This reaction was proved to be oxygen-dependent.

The reaction RH + $O_2$ --> ROOH (See Fig. 2.7), is unlikely to occur since the unsaturated fatty acid (RH) and the hydroperoxide (ROOH) are in the singlet states whereas the oxygen ($O_2$) is in the triplet state -- hence lack of conservation of spin (Aurand *et al.*, 1977). Spin conservation would be satisfied if the oxygen was in the singlet state, as can be achieved by chemical, enzymatic, or sensitized photooxidation. Since it was found that riboflavin could promote photodecomposition in singlet mediated reactions, Aurand and coworkers (1977) proposed the following mechanisms:

$$Sen + hv \rightarrow {}^1Sen^* \rightarrow {}^3Sen^*$$

$${}^3Sen^* + {}^3O_2 \rightarrow {}^1Sen + {}^1O_2{}^*$$

$${}^1O_2 + RH \rightarrow ROOH \rightarrow \text{Photo-oxidation Products}$$

(Sen = Riboflavin, which acts as a sensitizer)

It was postulated that riboflavin could generate singlet oxygen via the excited triplet state. Other workers have theorized methional to be the major component responsible for the burnt flavour produced when milk is exposed to sunlight (Allen and Parks, 1975; Shipe, 1980). Methional is said to be a product of the Strecker degradation of methionine (note: dicarbonyl required for reaction):

$CH_3SCH_2CH_2CHNH_2COOH \rightarrow CH_3SCH_2CH_2CHO + NH_3 + CO_2$

Photo-induced lipid oxidation represents the other category of light-induced reactions and is very similar to autoxidation or metal-induced oxidized flavour. This off-flavour develops more slowly than the activated flavour and it has been proposed that homogenization enhances the light-activated component of this off-flavour, while inhibiting the oxidized component (Shipe, 1980).
The present widespread use of fluorescent illumination of milk in display cases has been known to cause light-induced oxidized flavour, particularly in plastic and blow mould containers (Barnard, 1973). Also, it has been reported that nutritional losses (ascorbic acid and riboflavin) occur when milk in paperboard and plastic containers were exposed to fluorescent light for various times (Hansen *et al.*, 1975; Hedrick & Glass, 1975).

Several factors may influence the progress of photochemical reactions in milk. These include light source (wavelength and intensity), exposure time, oxygen, temperature and packaging material.

### 2.4.7 Effect of acidity on oxidation

This parameter may have an effect on the susceptibility of milk to oxidation, although it has not been studied thoroughly (Parks, 1980). Early experiments indicated that an increase in pH of 0.1 was enough to inhibit oxidation in fluid milk for 24 hours. Copper has been implicated in the

development of oxidized flavours in dairy products such as butter and ice-cream (Parks, 1980).

### 2.4.8 Oxidation as affected by homogenization

During homogenization, the total milk fat globule surface increases several-fold and a lipid-casein complex is formed at the globule surface (Karel, 1973). According to Fox *et al.* (1960), this complex formation entails distortion of the casein micelles by homogenization pressure, as well as the increase in globule area. Naturally, when the nature of the membrane changes, drastic differences in the behaviour of milk can also be expected. These changes may be manifested by (a) tendency for gelling, (b) a relative resistance to metal or photocatalyzed reactions and (c) susceptibility to enzyme catalyzed reactions leading to peroxidation (Shahani, 1974; Karel, 1973).

### 2.4.9 Lipolysis

Lipolysis has been defined by Downey (1974), as the enzymic hydrolysis of milk fat triglycerides. This reaction has great economic significance, in that accumulation of its reaction products (free fatty acids) is responsible for the lipolytic off-flavour known as hydrolytic rancidity. Although several mechanical and inherent physiological processes can result in lipolysis, the FGM effectively protects the milk triglycerides from attack by lipolytic enzymes present in normal milk (Downey and Murphy, 1975;

Jensen, 1964). In addition, association of these enzymes with the casein micelles and the possible presence of lipolytic inhibitors also contribute to the relative high resistance to enzyme attack.

Normal milk contains at least two naturally present lipases, one of which is associated with spontaneous lipolysis. The other (plasma lipase) remains loosely bound to the casein micelle until adsorbed onto the milk fat globule by an activation process (Jensen, 1964). Lipase has been found to be inactivated by oxidation, and to be sensitive to copper and iron (Shipe, 1980). The explanation offered for these observations was that lipase contains sulphydryl groups which are associated with enzyme activity.

Activation of lipase can occur via homogenization, shaking and temperature manipulations, eg. cooling to 5°C, then warming to 30°C, and finally cooling to 5°C (Chandan and Shahani, 1964). In the case of homogenization, activation may be rapid, due to an increase in substrate surface area and a recoating of the newly formed globules with lipase-containing casein (Jensen, 1964). Temperature changes such as cooling, may disrupt the FGM or alter the association of the lipase with other milk constituents (Shipe, 1980). Generally, activation may occur by:

i. Facilitating the release of lipase from the casein micelle

ii. Promoting the adsorption of enzyme on the fat globule

iii. Altering the orientation of the adsorbed fat globule membrane (Chandan and Shahani, 1964).

Agitation, particularly when accompanied with foaming, has been shown to cause a redistribution of the enzyme between the cream and the skim milk phase (Deeth and Fitz-Gerald, 1977). The accumulation of mono-, di-glycerides, and free fatty acids that results when milk lipase is in contact with milk fat, confers a rancid flavour on the milk. Chemically, lipolyzed flavour is primarily due to the $C_4$ - $C_{12}$ volatile fatty acids. The lipase does not differentiate between short and long chain fatty acids attached to the primary positions of the same glycerol, however, since butyrate appears mostly to be a primary ester, an apparent preferential release of butyrate occurs (Shipe, 1980). Once induced by agitation, lipolysis proceeds rapidly only for a relatively short period, after which it levels off, with no further accumulation of free fatty acids. This phenomenon may be due to the build-up of inhibitory free fatty acids at the fat globule interface, and failure of the enzymes to desorb from the interface, leading to a gradual inhibition of lipolysis (Downey, 1980).

The extent of activation has been found to increase with the duration and degree of agitation, while it decreases with the age of the milk at agitation (Tarrasuk and Frankel, 1955; Kitchen and Aston, 1970). Maximum activation has been reported to take place at temperatures

ranging between 40°C and 50°C (Fitz-Gerald, 1974), whereas, Kitchen and Aston (1970), proposed a slightly lower temperature (37°C).

As milks from individual cows are known to differ in their propensity to develop lipolytic flavours, it is customary to distinguish between naturally active milk from normal milk. Causes of spontaneous lipolysis are not fully understood, although it has been postulated that the level of phospholipid containing substances in the milk is important (Driessen and Stadhouders, 1974). Review studies carried out by Deeth and Fitz-Gerald (1976), however attributed spontaneous lipolysis to several physiological factors such as blood-derived constituents, late lactation, poor nutrition (particularly during late lactation), low milk yield, oestrus cycle and increased cell count.

In an effort to control induced lipolysis, milk should be cooled immediately after milking and with minimum agitation, prior to pasteurization (Shipe 1980). Also, it has been suggested that weigh jars (which inevitably cause lipolysis), should be disconnected during milking when weights are not being recorded (Pillay *et al.*, 1980b).

### 2.4.10 Measurement of lipolysis

The extent of lipolysis occurring in milk systems can be followed by the determination of the Acid Degree Value (ADV), which assays for the free fatty acids present (Marth, 1978). According to Thomas *et al.* (1955), good raw milk has

an ADV not greater than 0.9. The onset of activation can be recognized by an increase in ADV to 1.5 or greater. More recent studies on the threshold ADV for lipolyzed milk (as detectable by sensory analysis), indicate that in estimation by the standard BDI method (Marth, 1978), values lie between 1.85 and 2.05, whereas by the modified Frankel and Tarrasuk method, the threshold value lies between 4.1 and 4.5 (Pillay *et al.*, 1980a), hence the definite boundaries specified by Thomas *et al.* (1955) seem questionable.

The butterfat changes that may occur in milk can be influenced by feeding protected lipid supplements. As discussed in Section 2.2.1, there is usually an increase in the polyunsaturated fatty acids produced, and a compensatory decrease in short chain fatty acids with high levels of PLFS supplementation. This inevitably caused a decrease in the oxidative stability of the milk produced (Haase, 1977). The information on the effects of feeding low levels of PLFS and the corresponding susceptibility of milk and butter to oxidation and lipolysis is scant, hence the inception of this study.

## 3. OBJECTIVES AND EXPERIMENTAL DESIGN

The main objective of this investigation was to determine the effects of feeding commercially realistic levels of canola-based "Protec" to lactating dairy cows on the quality of milk and butter. Three major experiments were carried out.

1. A threshold study to determine the level at which changes are first detectable in the quality of milk and butter.
2. To determine the susceptibility of "Protec" itself to oxidative changes during storage and the effect of feeding the stored "Protec" on milk and butter quality.
3. The evaluation of the milk and butter produced from commercial dairy herds with a history of continued use of "Protec".

The first two experiments were carried out in collaboration with the University of Alberta Dairy Research Unit (operated by the Department of Animal Scence). Commercial farms in the vicinity of Edmonton were utilized in the third experiment.

### 3.1 Experiment 1 - Determination of PLFS Threshold

*Objective.* A threshold study was conducted to determine the lowest level of "Protec" supplementation which would cause detectable changes in milk and butter quality, as determined by sensory, chemical and physical tests (Table 3.1).

*Experimental Design.* Isonitrogenous diets (Table 3.2)

Table 3.1 Analytical Procedures performed on Milk and Butter

| | Milk | | |
|---|---|---|---|
| | From Individual Cows | From Pairs of Cows Fed the Same Diet | Butter |
| Composition | | | |
| Butterfat | x | x | x |
| Protein | x | x | - |
| Lactose | x | x | - |
| Total Solids | x | x | - |
| Solids-non-fat | x | x | - |
| Iodine Value | - | - | x |
| Functional Properties | | | |
| Penetrometry | - | - | x |
| Viscosimetry | - | - | x |
| Dropping Point | - | - | x |
| Solid Fat Content | - | - | x |
| Rancidity and Oxidation | | | |
| ADV | - | x | - |
| TBA | - | x | - |
| Peroxide Value | - | - | x |
| Flavour | | | |
| Expert Evaluation | - | x | x |
| Triangle Test | - | x | x |
| Signal Detection Test | - | - | x |

Table 3.2 Experiment 1. Formulation of Test Concentrates (%)

| | % Protec | | | |
|---|---|---|---|---|
| | 0 | 3 | 6 | 9 |
| Diet number | 1 | 2 | 3 | 4 |
| Protec | 0 | 3 | 6 | 9 |
| Rapeseed meal | 21 | 20 | 19 | 18 |
| Rolled oats | 25 | 25 | 25 | 25 |
| Rolled barley | 42 | 40 | 38 | 36 |
| Rolled wheat | 5 | 5 | 5 | 5 |
| Molasses | 3 | 3 | 3 | 3 |
| Calcium phosphate | 1.2 | 1.2 | 1.2 | 1.2 |
| Limestone | 1.4 | 1.4 | 1.4 | 1.4 |
| Non med. trace salt | 1.0 | 1.0 | 1.0 | 1.0 |
| Vit. A-D-E | 0.05 | 0.05 | 0.05 | 0.05 |
| Vit. $D^3$ | 0.03 | 0.03 | 0.03 | 0.03 |

formulated to contain graded levels (0, 3, 6, 9%) of "Protec", were fed to eight Holstein dairy cows in their early to mid-lactation periods. A double 4 x 4 Latin Square design was employed, where two cows were fed the same diet for three weeks in each trial. The entire experiment was twelve weeks in duration. Milk was collected, processed, and treated after three weeks (i.e. at the end of each trial), using methods described in Section 4.2 and 4.3. Finished products (milk and butter) were evaluated by sensory methods and analyzed by chemical and physical tests (Section 4.5 and 4.6)

**3.2 Experiment 2 - Storage Stability of "Protec" and Effect of Feeding Stored "Protec" on Milk and Butter Quality**

*Objective.* The purpose of this study was two-fold:

(a) To assess the susceptibility of commercially manufactured "Protec" to oxidative changes during controlled storage.

(b) To evaluate whether the quality of milk and butter would be affected by feeding the threshold level (as determined in Expt. 1) of stored "Protec" to lactating dairy cows.

*Experimental Design.* 500g of "Protec" were incubated at 4°C, 20°C (room temperature), and 40°C, for a period of twelve weeks. Subsamples were taken at two week intervals and analyzed for oxidative changes as indicated by the Peroxide

Value Test. Concurrently, 80kg of "Protec" were stored in open containers at 40°C for twelve weeks. The resulting stored "Protec" was supplemented in diets to determine whether the effects of storage would appear as off-flavours in milk and butter. Diets containing no "Protec", 6% fresh "Protec" and 6% stored "Protec" were fed in a completely randomized design, to twelve Holstein dairy cows in late lactation for three weeks. At the end of the feeding trial, milk from the four cows being fed each diet was combined, processed and analyzed. The composition and quality of homogenized, pasteurized milks and butters produced were checked by chemical, physical and sensory tests (Sections 4.5 and 4.6).

### 3.3 Experiment 3 - Evaluation of Milk and Butter from Cows being fed "Protec" in Commercial Dairy Herds

*Objective.* This study was concerned with the assessment of the quality of milk and butter when produced from commercial dairy herds having a continued history of PLFS use.

*Experimental Design.* Milk was collected from two commercial dairy farms that had been using protected lipid feed supplements for at least three years, and also from two commercial dairy farms that had never used "Protec", or any other PLFS. All farms concerned had herds consisting of Holstein dairy cows. For comparison, milk was also obtained

from a commercial dairy manufacturing plant in Edmonton, Alberta: A portion of milks obtained from herds being fed "Protec" was combined, homogenized and pasteurized for sensory evaluation (Section 4.6.1), while the remainder was processed into butter. Sensory evaluation and tests related to butter hardness were carried out as in previous experiments. Comparisons were made with milks and butters processed in a similar manner which were obtained from herds not being fed "Protec".

# 4. MATERIALS AND METHODS

## 4.1 Raw Materials

### 4.1.1 Protected Lipid Feed Supplement

The supplement used throughout this study was the canola-based product, "Protec". This was manufactured (Fig. 4.1) and supplied by Barrhead Alfafa and Protec Products Ltd., Barrhead, Alberta. In this process (personal observation), the canola seeds and meal are crushed in a plate mill prior to emulsification with sodium hydroxide. The slurry that results at this stage is drum dried, packaged and distributed to dairy farmers on a demand-basis.

### 4.1.2 Microstructure of "Protec"

The microstructure of the unprotected and protected canola was examined as described by Stanley *et al.* (1976). Dehulled seeds or granules of the protected lipid material were prefixed in a 3% glutaraldehyde solution (in pH 7.0 buffer). After post-fixing in 1% $KMnO_4$ at 5°C , the seeds were rinsed in several changes of distilled water. Dehydration in seven changes of ethanol preceded air drying and mounting. The scanning electron microscopy was carried out in the Department of Entomology on both unprotected and protected canola. A typical micrograph (Plate 4.1-B&D) illustrates the distribution of canola meal on the protected

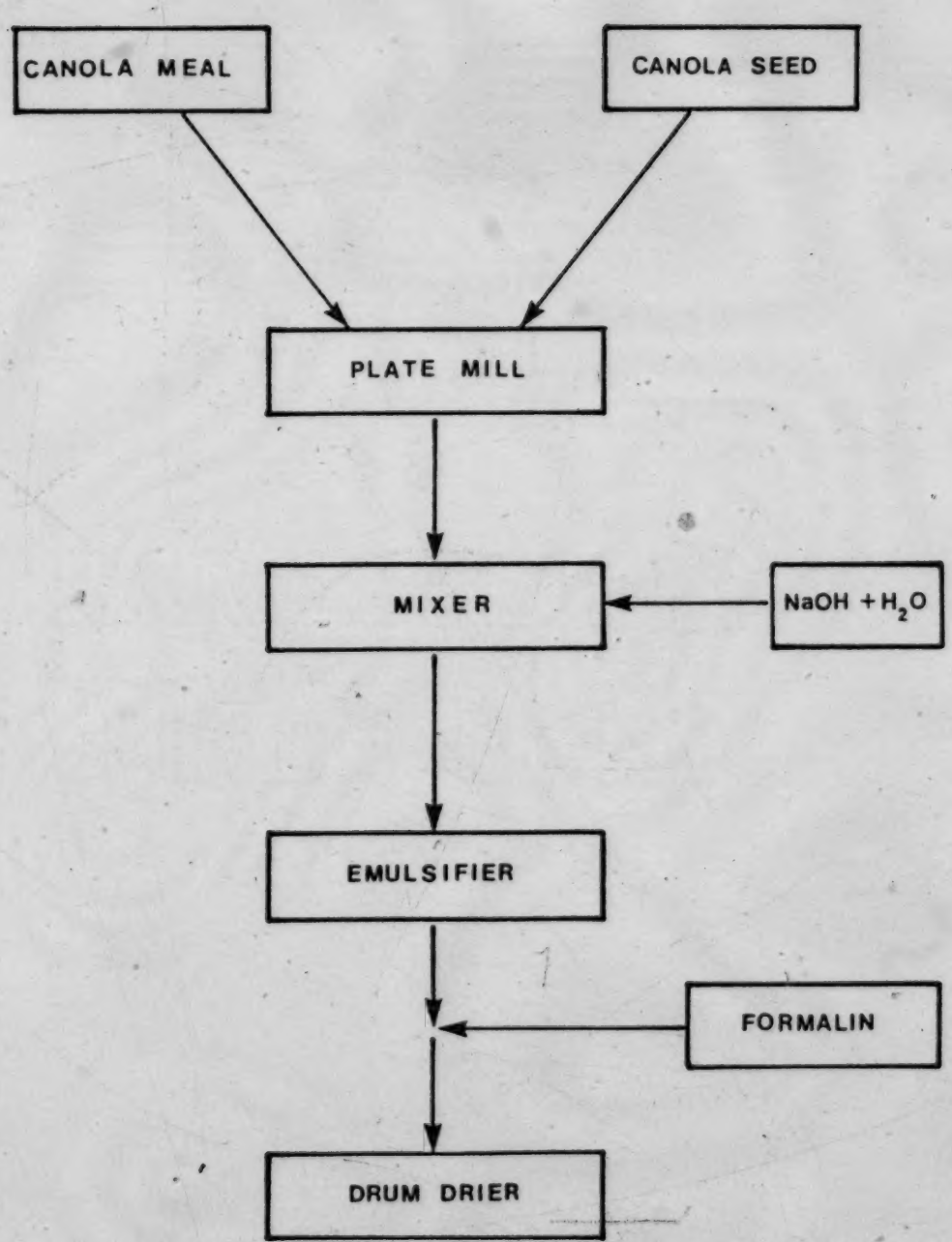



Fig. 4.1 Diagram of 'Protec' Manufacture

canola seed.

### 4.1.3 Formulated Diets

The test diets were based on the standard rolled ration fed at the University of Alberta Dairy Farm (Table 4.1). Standard procedures of the University Farm operations were followed; this included pelleting of the experimental concentrates in a Pellet Mill subsequent to mixing in a 1 Ton Batch Mixer. The standard diet used consisted of 7 kg hay, 3 kg beet pulp and 14 kg concentrate. In addition, water and a mixture of equal parts of trace mineral salt and calcium phosphate were offered *ad libitum*. The actual amounts of hay and concentrate offered varied slightly with the past production record of individual cows. Diets were stored at a cool temperature in the store-room at the farm.

### 4.1.4 "Stored Protec"

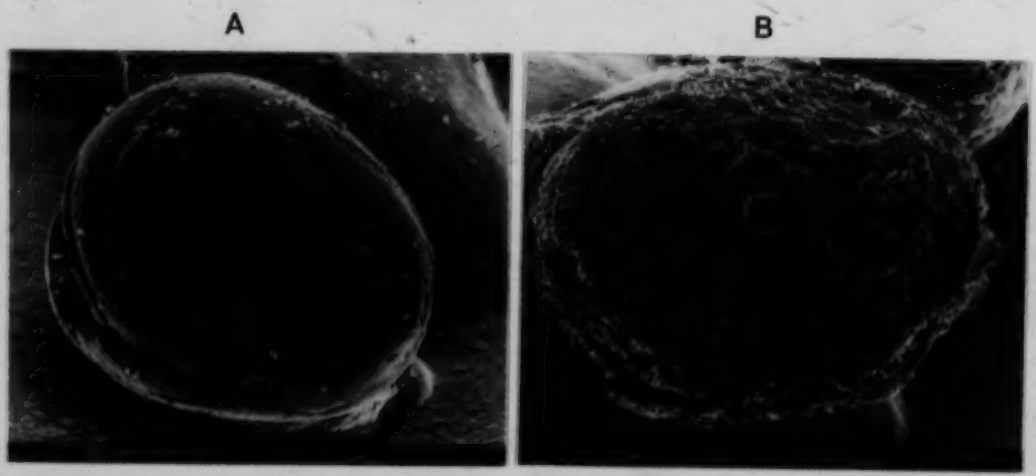
In order to assess the susceptibility of fresh "Protec" to oxidative changes during storage, "Protec" was incubated in open containers at 40°C for a period of twelve weeks. The product resulting is referred to as "Stored Protec" throughout this work.

Table 4.1 Standard Rolled Ration (Control #1) Fed By University of Alberta Dairy Research Unit

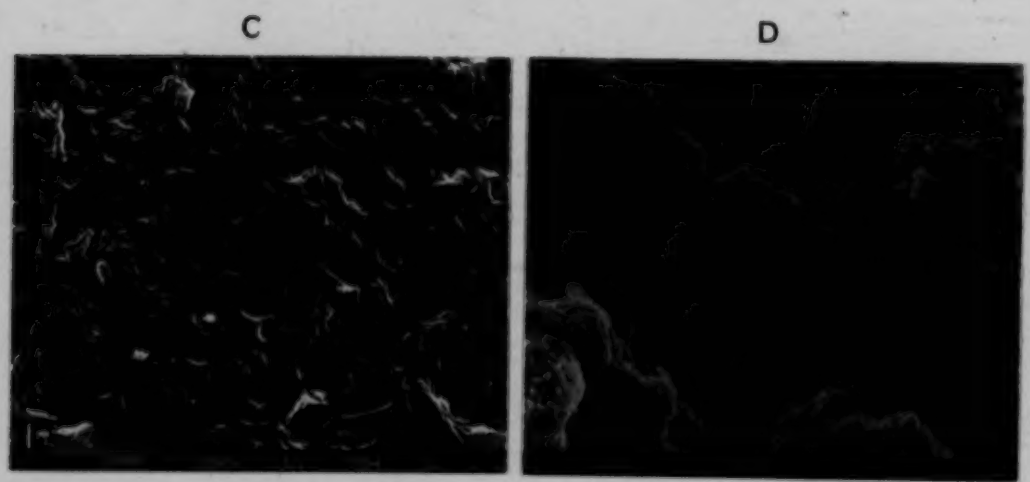
| | |
|---|---|
| Rapeseed meal[1] | 20 |
| Rolled oats | 25 |
| Rolled barley | 43 |
| Rolled wheat | 5 |
| Molasses | 3 |
| Calcium phosphate[1] | 1.2 |
| Limestone[1] | 1.4 |
| Non med. trace salt[1] | 1.0 |
| Vit. A-D-E[1] | 0.05 |
| Vit. $D^3$ [1] | 0.03 |

[1]Pelleted.

Plate 4.1




Scanning Electron Micrograph (SEM) of unprotected (A) and protected (B) canola seed - low magnification (x27.5 and x25 resp.).




Scanning Electron Micrograph (SEM) of unprotected (C) and protected (D) canola seed - high magnification (x750 and x800 resp.).

## 4.2 Processing

### 4.2.1 Collection of Milk

Milk from the University Dairy Farm was collected directly from the milk receiver of the pipeline milking equipment into stainless steel milk cans, and transported to the Department of Food Science, University of Alberta. Collection of milk was made twice per day during the hours of 7:00 am - 8:30 am and 3:00 pm - 4:30 pm, when cows were normally milked at the farm. Milk from commercial dairy operations was obtained from a refrigerated bulk tank located on each farm. All milks were cooled quickly to about 8°C by immersing cans in a cheese vat containing ice water and by stirring the contents of each can intermittently. After cooling, the milk was stored at 4°C for processing.

### 4.2.2 Homogenization of Milk

Morning and afternoon milkings (comprising a production day) were obtained from cows being fed the same diet, then pooled and thoroughly mixed. A 15-20 litre sample was taken from each batch for homogenization, while the remainder was refrigerated and used in cream production the following day. After heating to 50°C , the respective milks were passed through a Gaulin Laboratory Homogenizer, Model 15M - 8TA (Everett, Mass.) using a pressure of 3000 psi. The individual batches of homogenized milk were pasteurized immediately. The homogenizer was cleaned by the Cleaning in

Place (CIP) method and rinsed with hot water preceding each run.

### 4.2.3 Pasteurization of Milk

Homogenized milks were batch-pasteurized in a laboratory stainless steel double-jacketed kettle. The milk was held at 73°C for thirty (30) seconds, then quickly cooled to ca 10°C by passage of cold water through the jacket of the kettle and by immersion in ice water (Section 4.2.1). Storage at 4°C followed.

### 4.2.4 Separation of Cream

The morning and afternoon milk of the second day's milking, collected from cows on the same diet was pooled and combined with the milk remaining from the previous day's milking. The resulting composite was heated to 50°C while stirring continuously using a mechanical stirrer. Cream containing at least 50% fat (Table 4.2) was obtained by separation on a Liquid-Liquid-Solid Disc (#58) Pilot Plant Centrifuge, Alfa-Laval (De Laval Company Ltd., Peterborough, Ont.). The cream was batch-pasteurized at 75°C for 30 minutes, sampled for analysis and cooled overnight at 4°C .

### 4.2.5 Butter-making

Creams were churned into butter in a laboratory Hobart Mixer Kitchen Aid - Model K5-A (Troy, Ohio) using a pronged blade. The Hobart bowl was surrounded with ice throughout

Table 4.2 Fat Content of Creams Used in Buttermaking

| | | % Butterfat[1] |
|---|---|---|
| Experiment 1 | | |
| A. | 0% Protec | 60 |
| | 3% Protec | 64 |
| | 6% Protec | 56 |
| | 9% Protec | 60 |
| B. | 0% Protec | 71 |
| | 3% Protec | 54 |
| | 6% Protec | 70 |
| | 9% Protec | 73 |
| C. | 0% Protec | >50[2] |
| | 3% Protec | >50[2] |
| | 6% Protec | >50[2] |
| | 9% Protec | >50[2] |
| D. | 0% Protec | 68 |
| | 3% Protec | 69 |
| | 6% Protec | 71 |
| | 9% Protec | 60 |
| Experiment 2 | | |
| | 0% Protec | 72 |
| | 6% Fresh Protec | 67 |
| | 6% Stored Protec | 68 |
| Experiment 3 | | |
| | Control (Without Protec) A | 77 |
| | B | 76 |
| | Test (With Protec) A | 72 |
| | B | 75 |

[1]Babcock procedure.

[2]Insufficient sample to repeat analysis.

churning to ensure that the cream remained chilled. Buttermilk was washed from the butter with cold water. The excess moisture was removed by draining and subsequently working the butter by hand manipulations. Butters were packed in 250 ml plastic containers and stored for further use.

### 4.2.6 Storage of Milk and Butter

Following homogenization and pasteurization, milks were stored in amber 1-litre-glass bottles at 4°C . Butter samples not required for immediate use were frozen under nitrogen at -40°C . Other butters were refrigerated at 4°C .

## 4.3 Susceptibility of Milk to Induced Oxidative and Hydrolytic Rancidity

### 4.3.1 Copper-induced oxidation of Milk

Copper sulphate solution was added to one-litre samples of homogenized and pasteurized milk in order to attain a final concentration of 1 ppm copper (Shipe *et al.*, 1978). Samples were kept in amber glass bottles at 4°C for at least 24 hours prior to analysis (Section 4.5.1).

### 4.3.2 Exposure of Milk to Fluorescent Light

Homogenized and pasteurized milks were placed in 1-litre glass Erlenmeyer Flasks and sealed with Saran Wrap. The flasks were positioned such that the surface of the milk was 35 cm under a commercial "Bright-Stik" lamp (Appendix B). Samples were exposed to 300-550 lux intensity for 3 and 5 days at 4°C before analysing (Section 4.5.1).

### 4.3.3 Inducement of Hydrolytic Rancidity in Milk

Raw milk samples (ca 250ml) were warmed to 37°C in a water bath and immediately whipped in a Waring Blender for 60 seconds. The milks were then incubated for 15, 30, and 60 minutes at 37°C prior to pasteurization at 73°C for 30 seconds. Refrigeration at 4°C followed.

### 4.3.4 Oxidative Stability of Butter

Covered plastic containers with butter samples were stored at 4°C for 30 days in order to determine the susceptibility of butters to oxidative rancidity as measured by the Peroxide Value test (Section 4.5.2).

## 4.4 Proximate Analyses

Unless otherwise stated, all determinations were performed in duplicate.

### 4.4.1 Diets

Pooled samples of test concentrates fed to cows during the first feeding trial resulted in four diet mixes containing 0, 3, 6, and 9% "Protec". Samples were ground in a coffee mill and analyzed for moisture, crude protein, ether extract, gross energy and dry matter. With the exception of dry matter, all results were reported on a dry basis.

*Moisture.* Moisture was determined by a standard AOAC (1980) procedure (Method 7.003). Samples were dried to constant weight in a vacuum oven at 95-100°C.

*Crude Protein.* Crude protein was measured according to the Kjeldahl procedure (AOAC, 1980, Method 7.016). Protein content was calculated as (%N x 6.25).

*Crude Fat (Ether Extract).* The fat content of the respective diets was determined by quantitative extraction using anhydrous ether in a Soxhlet apparatus. The extraction period was 5 hours at a condensation rate of 5-6 drops/second (AOAC, 1980, Method 7.056).

*Gross Energy.* The gross energy of the diet was determined according to the AOAC Method, 1980. A Parr Oxygen Adiabatic Bomb Calorimeter (Model 1241) equipped with a Parr Calorimeter Master Control (Model 1680) was used when available to provide direct energy values. A Parr Calorimeter (Model 1241) was also used in conjunction with a Parr Calorimetric Thermometer (Model 15557). This required manual calculation of the energy content.

### 4.4.2 Milk

The following compositional analyses were carried out by staff of the Central Milk Testing division at the O.S Longman Building, Edmonton,Alberta. Standard methods were used.

*Fat*. The fat content of the raw milks was determined by two methods. The Mojonnier method was according to a modified AOAC method (AOAC, 1980; Method 16.082), where the fat was dried at 135 °C at 20 Hg for 5 min. An automated procedure based on infra-red analysis (AOAC, 1980) was also used.

*Protein and Lactose*. Both protein and lactose were determined using the Milkoscan Infra-red Milk Analyzer (AOAC, 1980; Methods 16.084 and 16.086 resp.).

*Total Solids*. Duplicate samples of 3.0-3.5 ml of raw milk were predried for 30 minutes on a steam bath, subsequent to being placed in a forced air oven at 100°C. The residues were dried to a constant weight (AOAC, 1980; Method 16.032).

*Solids Non Fat*. The solids non fat (SNF) values were

$$\% \text{ SNF} = \% \text{ Total Solids} - \% \text{ Fat}$$

### 4.4.3 Butter

Preceding analyses, butters were softened to a creamy consistency by warming in a water bath (39°C), with intermittent shaking (AOAC, 1980; Method 16.204).

*Moisture*. All butter samples were tested in triplicate for moisture using the AOAC (1980) Method 16.205. The creamy butter sample was dried to a constant weight in an oven at

the temperature of boiling water.

*Fat*. The fat content of butters was determined according to the Direct AOAC (1980) Method 16.207, where fat resulting from moisture analysis was extracted with petroleum ether (BP 35-60 and evaporated to a constant weight.

### 4.5 Chemical and Physical Analyses of Milkfat

Analyses were carried out in duplicate unless otherwise stated.

#### 4.5.1 Oxidative Stability by TBA Test

The TBA method of King, 1962, was employed on homogenized and pasteurized milk. Milk proteins were precipitated with trichloroacetic acid and filtered. A portion of the filtrate was mixed with the TBA reagent. The colour was developed and the absorbancy of the solution measured at 532 nm. Susceptibility of milks to induced oxidative changes was evaluated by subjecting Cu-oxidised milk and milk exposed to fluorescent light (Section 4.3.1 & 4.3.2) to the TBA test.

#### 4.5.2 Oxidative Stability by Peroxide Value Determination

The AOCS (1980) procedure (Cd 8-53) was used with the following precautions and modifications :

a) The weight of the oil sample was kept as close as possible to 5.000g;

b) Light was kept to a minimum in the test room;

c) Holding time in the dark after addition of acetic acid/chloroform mixture was ten minutes and fifteen minutes after the addition of saturated potassium iodide;

d) Exactly 0.5 ml starch indicator was added at the start of the titration procedure.

The excess iodine liberated in the reaction of the butteroil with saturated potassium iodide solution was titrated with 0.01 N sodium thiosulphate.

$$\text{Peroxide Value} = 2 \times \frac{(S-B)(N)(1000)}{\text{Weight of Oil}}$$

where S = Titration of butteroil in ml
B = Titration of blank in ml
N = Normality of sodium thiosulphate

### 4.5.3 Determination of Free Fatty Acid Content

The free fatty acid content of the milks was determined using the standard method for evaluation of hydrolytic rancidity (Marth, 1978), a modification being that the fat was weighed rather than measured. The milk fat was extracted with BDI Reagent (surfactant) and the free fatty acid level of the raw milk was measured by the Acid Degree Value (ADV) as determined by:

$$\text{ADV} = \frac{[\text{ml KOH(s)} - \text{ml KOH(b)}](N)(100)}{\text{Weight of milkfat}}$$

where ml KOH(s) = Titration of sample with KOH
ml KOH(b) = Titration of blank with KOH
N = Normality of Potassium Hydroxide (KOH)

The susceptibility of milk to induced hydrolytic rancidity was elucidated by the determination of the ADV of the treated milk as prepared in Section 4.3.3.

### 4.5.4 Level of Unsaturation

The Iodine Value was used as the index of the level of unsaturation in butterfat. Determination of the Iodine Value (IV) was carried out on butteroil as described in the AOCS Method, 1980. The butteroil was produced by filtering melted butter through a Whatman No. 4 filter paper at 40°C in an air convection oven.

$$IV = \frac{(B-S)(N)(12.69)}{\text{Weight of butteroil}}$$

where B = Titration of blank
S = Titration of sample
N = Normality of sodium thiosulphate

### 4.5.5 Hardness

Subsamples of butters produced (Section 4.2.5) were packed in open-ended stainless steel cylinders, 50 mm in diameter and 29 mm in height, prior to pentrometry determinations. In order to ensure proper equilibration with temperature, butters were stored in the chamber at 12°C for 24 hours (Amer and Myhr, 1973). The flat disc penetrometer(approximately 14 mm in diameter), Minarik Electronic Co., Model SL 14 (Los Angeles, Cal.) was used at

a constant temperature (12°C) in a Modu-Lab Room, Ser. No. 138, Labline (Chicago, Ill.). The probe of the penetrometer was adjusted to descend at a constant speed into the butter sample, and the resulting force-distance plot was obtained on a Honeywell recorder, Electronik 19 (Philadelpha, Pa.). The peak force was noted and readings were converted to hardness values in $kg/cm^2$. Four penetrometer readings were made in each cylinder and the results were averaged.

### 4.5.6 Softening Point

The softening point of butter was obtained by viscometry measurements carried out on a Haake Rotovisco RV3 (Berlin, Germany) as described by Wong *et al.*, 1982. The measuring apparatus used was the NV sensor system which is comprised of a coaxial cylinder in an enclosed tempering container.

Approximately 7.4 g of butter at 35°C were placed in the sample cup prior to programmed heating between 20°C and 50°C. The temperature-voltage plots were recorded by a Linear Instruments Recorder, Model 300, Cole-Parmer, (Chicago, Ill.). The softening point was measured as the adjusted intersection of the temperature and voltage curves, and was reported as °C.

### 4.5.7 Dropping Point

The dropping point of butterfat estimates the point at which the fat is fluid enough to flow through a specified aperture. An indication of the melting characteristics of fats can therefore be obtained from this determination. Analyses were carried out on a Mettler FP3 Automatic Dropping Point apparatus (Mettler Instrument Corp., Princeton, N.J.), according the method of Mertens and DeMan (1972a). Butters were melted and filtered before filling prechilled sample cups. The heating rate on duplicate samples was 2°C per minute.

### 4.5.8 Oiling Off

The quantity of free fat exuded at 20°C was used to indicate the extent of oiling off occurring in butter samples. The method employed was a modification of the Mortensen and Danmark (1980) procedure. A plug of butter with a diameter of 9 mm and a height of 15 mm was sampled from butters stored at 4°C for at least 24 hours. The plug of butter was placed in the centre of a Whatman No. 1 type filter paper (9 cm in diameter) prior to placing in a thermostat at 20°C. The amount of fat absorbed in the filter paper after 4 hours was expressed in % as w/w of the initial quantity of butter used.

### 4.5.9 Solid Fat content

The solid fat content of single samples of butters was determined by the procedure of Mertens and DeMan (1972b). The instrument used was the Newport NMR analyzer, type MK1. The reference temperature in all cases was 60°C and the reference sample used was olive oil. Tempering of the samples was critical. The % solid fat was determined at each temperature by:

$$\% \text{ Solid Fat} = 1 - \frac{\text{SS at T°C / SS at 60°C}}{\text{RS at T°C / RS at 60°C}} \times 100$$

(SS and RS are sample and reference signals, respectively)

### 4.6 Sensory Evaluation of Milk

Sensory analyses of homogenized and pasteurized milk were conducted at the end of each feeding period to assess if organoleptic quality changes occurred as a result of feeding protected lipid feed supplement. In the threshold study (Experiment 1), where a Latin Square design was used, the triangle test (Larmond, 1977) was employed to identify differences in the milk produced. The degree of difference (slight, moderate, much, extreme) was noted by untrained panelists (Appendix C). The untrained panel consisted of men and women between the ages of 20 and 50, randomly selected from staff and student members of the Department of Food Science, University of Alberta. The same panel was used throughout the study with the numbers of panelists available

varying somewhat.

All sensory evaluations took place in individual tasting booths, which were illuminated with incandescent light. Morning sessions (10:00 - 12:00 am) were used since they were found to be most convenient.

Milk was equilibrated to room temperature for approximately two hours before serving in 30 ml polyethelene cups to panelists. Test samples were identified by three-digit random numbers and the participants were instructed as to the order in which the milk were to be tasted. Tap water was provided for mouth rinsing.

Triangle tests were also conducted to determine whether the feeding of stored, oxidized "Protec" would be detected as off-flavours in the milk, and to detect differences in milk flavour when diets containing "Protec" were fed to commercial dairy herds. The conditions previously described were implemented.

In addition, a semi-trained panel consisting of 1-2 students and a "coach" of the University of Alberta dairy judging team, (all of whom had previous judging experience in the critical evaluation of dairy products), examined the milk in afternoon sessions. Off-flavours, if present, were identified as "slight", "definite", and "pronounced" according to the American Dairy Science Association (ADSA) milk score cards. Samples of milk stored in amber glass bottles at 4°C for 10 days were evaluated similarly.

## 4.7 Sensory Evaluation of Butter

Differences in the quality of butters produced as a consequence of feeding "Protec" were corroborated by sensory evaluation.

Butters made in the threshold study were evaluated according to the Triangle Test (Larmond, 1977), panelists and conditions being as described in Section 4.6.1.

Initially, a small portion of butter was placed on unsalted crackers and served to 18-24 untrained panelists. A knife was provided. In subsequent trials, butter was placed in 30 ml polyethylene containers in order to eliminate the flavour of the crackers. The type of difference (spreadability, taste, both) was recorded on score sheets (Appendix D).

The triangle test was also employed to note if changes in butter quality occurred when stored "Protec" was fed to the dairy cows. However, in order to compare flavour differences only, inconsistencies with respect to butter softness were removed by whipping each sample for 5 minutes in a Kitchen Aid mixer. Whipped butters were evaluated using the Signal Detection Test (Mahoney, *et al.*, 1979).

One reference butter and three test butters were offered in 30 ml polyethelene cups with a spoon. The participants were required to indicate whether the test samples were the same or different from the reference sample. The degree of surety was recorded (Appendix E), and the probability of the panelists to correctly characterize

samples was calculated.

Butters produced from milk obtained from commercial dairy herds were evaluated for flavour differences by the Signal Detection Test as described above. One reference butter and two test butters were presented to untrained panelists for assessment.

The experienced panel previously used to test milk, also evaluated all unwhipped butters using ADSA butter score cards.

**4.8 Grading of Butter**

Butters from the threshold experiment (Expt. 1) and butters resulting when stored "Protec" was incorporated in the diets of lactating dairy cows were examined and graded in the standard manner by six Federal Butter Graders.

At the end of a routine examination of commercial dairy products, individual butters were presented to the Graders for assessment. Testers were seated at a large table located in a room lit with fluorescent light. Representative samples of butters were placed on paper plates identified with three-digit random numbers. Butter grades were recorded with additional comments.

# 5. RESULTS AND DISCUSSION

## 5.1 Experiment I: Effects of feeding graded levels of "Protec" on the quality of milk and butter

The purpose of this study was to determine whether a threshold level existed at which changes in milk and butter quality could be detected by chemical, physical and sensory tests. This was achieved by implementing a double 4x4 Latin Square design where eight lactating dairy cows were fed various diets for three weeks in each trial (Section 3.1).

### 5.1.1 Effect of feeding "Protec" on feed consumption, milk yield and milk composition of lactating dairy cows

The test concentrates were isonitrogenous (Table 5.1) as indicated by crude protein content of 14% dry basis. The amount of crude fat increased gradually from 2.88% at the 0% level of "Protec" supplementation, to 6.04% at the 9% level of supplementation, but only small differences were evidenced in gross energy, which rose from 3.5 kcal/g to 3.7 kcal/g as the level of "Protec" incorporation increased. Feed consumption patterns were not affected by feeding graded level (0,3,6 and 9%) of "Protec" to Holstein dairy cows ($p>0.05$; Table 5.2). No significant differences ($p>0.05$) were observed in milk yields, butterfat and protein yields of the pooled milks over the four periods, when 0, 3, 6, & 9% "Protec" was incorporated in the diets of dairy cows

Table 5.1. Expt I: Composition of Concentrate (Dry Basis)

| | % Protec | | | |
|---|---|---|---|---|
| | 0 | 3 | 6 | 9 |
| Crude protein (% N x 6.25) | 14.06 | 13.80 | 14.23 | 14.37 |
| Ether extract (%) | 2.88 | 3.81 | 4.78 | 6.04 |
| Gross energy (kcal/g) | 3.50 | 3.58 | 3.65 | 3.73 |

Table 5.2 Effect[1] of "Protec" on Feed Consumption, Milk Yield and Milk Composition of Lactating Dairy Cows

| | % Protec | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 3 | 6 | 9 | SE | $r^2$ |
| Feed consumption (kg/day) | | | | | | |
| Hay | 6.0 | 5.8 | 6.1 | 6.0 | 0.14 | 0.01 |
| Grain | 13.3 | 13.2 | 13.2 | 13.1 | 0.11 | -0.07 |
| Milk yield (kg/day) | | | | | | |
| Milk | 27.2$^{ab}$ | 26.4$^{b}$ | 28.7$^{a}$ | 27.5$^{ab}$ | 0.69 | 0.06 |
| FCM (4% BF)[3] | 19.0 | 19.8 | 20.3 | 19.9 | 0.68 | 0.09 |
| Butterfat[3] | 0.5 | 0.6 | 0.6 | 0.6 | 0.04 | 0.09 |
| Protein | 0.9 | 0.9 | 0.9 | 0.9 | 0.02 | 0.02 |
| Milk composition[4] (%) | | | | | | |
| Butterfat - Milkoscan | 1.9 | 2.3 | 2.1 | 2.0 | 0.17 | 0.03 |
| - Mojonnier | 2.0 | 2.4 | 2.2 | 2.2 | 0.17 | 0.05 |
| Protein | 3.3 | 3.3 | 3.2 | 3.2 | 0.03 | -0.15 |
| Lactose | 4.9 | 4.8 | 4.8 | 4.9 | 0.04 | 0.01 |
| Total solids | 10.8 | 11.2 | 10.8 | 11.0 | 0.16 | 0.02 |
| Solids-not-fat | 8.9 | 8.9 | 8.8 | 8.9 | 0.04 | -0.01 |

[1]Means within the same row followed by the same or no superscript are not significantly different ($P > 0.05$).

[2]Correlation with level of "Protec" in diet.

[3]Based on butterfat determined by the Mojonnier procedure.

[4]Averages ($n = 8$) of data for individual cows.

(Table 5.2). Although fat corrected milk (FCM) yields increased slightly from 19.0 kg/day when 0% "Protec" was fed, to 19.9 kg/day with the 9% "Protec" diet, the increase was not significant ($p>0.05$; Table 5.2 ).

The large variation that existed among cows is apparent when examining milk yields and butterfat levels of individual cows (Table 5.3). The trend for individual cows appeared to be a slight decrease in the milk yield with increasing levels of the "Protec" and a corresponding decrease in the butterfat content. This pattern was not apparent when pooled milks from cows on the same diet were analyzed, probably due to large standard deviations and coefficients of variation among individual cows (Table 5.3).

**5.1.2 Effect of "Protec" on the composition of milk and butter used in quality studies**

*Milk.* The composition of milk pooled from cows fed the same diet was not affected by the level of "Protec" in the diet. Similar levels of butterfat, protein, lactose, total solids, and solids-non-fat occurred in all milks ($p>0.05$; Table 5.4).

*Butter* Moisture and fat contents were generally similar (Table 5.4). However, butter from cows fed 6% "Protec" contained slightly more moisture than the other samples ($p>0.05$). All the butters (except one) passed the requirements of the Canadian Food and Drugs Act (1982) by containing at least 80% fat.

Table 5.3 Effect of "Protec" on Yield and Butterfat Levels of Milk From Individual Cows

| | Cow | % Protec 0 | 3 | 6 | 9 |
|---|---|---|---|---|---|
| Milk yield (kg/day) | | | | | |
| | 610 | 36.9 | 32.6 | 32.6 | 34.4 |
| | 613 | 34.4 | 36.9 | 35.1 | 32.6 |
| | 812 | 27.9 | 19.5 | 23.3 | 25.8 |
| | 824 | 26.4 | 28.0 | 32.7 | 25.9 |
| | 832 | 18.4 | 20.9 | 13.5 | 15.6 |
| | 833 | 23.5 | 18.4 | 28.0 | 21.4 |
| | 834 | 22.1 | 29.3 | 31.1 | 32.7 |
| | 841 | 27.8 | 30.6 | 33.1 | 31.9 |
| | Mean ± SD | 27.2±6.1 | 26.4±6.3 | 28.7±7.2 | 27.5±6.6 |
| | Coefficient of variation (%) | 22.4 | 23.9 | 25.1 | 24.0 |
| Butterfat[1] (%) | | | | | |
| | 610 | 1.84 | 1.50 | 1.50 | 1.42 |
| | 613 | 1.96 | 2.37 | 1.73 | 2.18 |
| | 812 | 1.41 | 4.24 | 2.96 | 2.97 |
| | 824 | 1.58 | 1.90 | 1.41 | 1.99 |
| | 832 | 2.40 | 3.02 | 3.47 | 2.64 |
| | 833 | 2.16 | 2.12 | 1.90 | 2.23 |
| | 834 | 2.23 | 1.76 | 2.10 | 1.49 |
| | 841 | 2.63 | 2.27 | 2.43 | 2.69 |
| | Mean ± SD | 2.03±0.41 | 2.41±0.86 | 2.19±0.72 | 2.20±0.56 |
| | Coefficient of variation (%) | 20.2 | 35.7 | 32.9 | 25.5 |

[1]Mojonnier procedure.

Table 5.4 Effect[1] of "Protec" on the Composition of Pooled Milks and Corresponding Butters

| | % Protec | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 3 | 6 | 9 | SE | $r^2$ |
| Milk (%) | | | | | | |
| Butterfat - Milkoscan | 2.1 | 2.1 | 1.9 | 2.1 | 0.08 | -0.07 |
| - Mojonnier | 2.2 | 2.2 | 2.0 | 2.2 | 0.09 | -0.02 |
| Protein | 3.3 | 3.2 | 3.2 | 3.2 | 0.02 | -0.32 |
| Lactose | 4.9 | 4.9 | 4.9 | 4.9 | 0.03 | 0.08 |
| Total solids | 11.0 | 11.0 | 10.8 | 11.0 | 0.10 | -0.12 |
| Solids-non-fat | 8.9 | 8.9 | 8.9 | 8.9 | 0.04 | -0.02 |
| Butter (%) | | | | | | |
| Moisture | $14.6^b$ | $14.9^b$ | $17.0^a$ | $15.5^b$ | 0.41 | -[3] |
| Fat | $82.6^a$ | $81.0^a$ | $79.9^b$ | $81.1^a$ | 0.47 | -[3] |

[1] Means within the same row followed by the same or no superscript are not significantly different ($p > 0.05$).

[2] Correlation with the level of "Protec" in the diet.

[3] Differences were likely due to processing techniques.

### 5.1.3 Sensory characteristics of milk

All milks were judged to be of acceptable quality and free from flavour defects before and after a ten day storage period at 4°C.

After each feeding trial, the untrained panel was asked to distinguish between milk from cow fed the control (0% "Protec") diet and the milk from cows fed each of the "Protec"-containing diets. At the 3% level of supplementation, panelists were not able to distinguish any flavour differences (Table 5.5) when compared with control milk (0% "Protec"). When presented with milks obtained at the 0% and 6% level of "Protec", panelists were able to identify a difference in milk flavour in only one trial out of four. At the 9% level of "Protec" in the diet, panelists distinguished a flavour difference in milks two out of four trials. The probabilities of correctly identifying the odd sample by chance were determined according to Roessler *et al.*, (1978), and analyzed as for a one-tailed test (Table 5.5). Difference between the comparison means for 3% and 9% "Protec" was significant ($p<0.05$),and the probability of correctly identifying the odd sample increased as the level of "Protec" increased. No objectionable flavours were noted. Possible factors such as the incorporation of canola-based PLFS in diets, milk handling procedures and the normal biological variation among cows may have contributed to the slight flavour differences noted.

Table 5.6 Expt I: Panelists Correctly Identifying Odd Milk Sample in Triangle Tests and Associated Levels of Significance[1]

| Comparison | Period A | Period B | Period C | Period D | Comparison $\bar{x}$ |
|---|---|---|---|---|---|
| 0 vs 3% PLFS | [illegible] (P=0.[illegible]) | 11/22 (P=0.079) | 8/17 (P=0.172) | 7/20 (P=0.521) | P = 0.28[a] |
| 0 vs 6% PLFS | 12/26 (P=0.121) | 9/22 (P=0.293) | 12/17 (P=0.002) | 10/20 (P=0.092) | P = 0.13[a,b] |
| 0 vs 9% PLFS | 13/26 (P=0.058) | 11/22 (P=0.079) | 12/17 (P=0.002) | 12/20 (P=0.013) | P = 0.04[b] |

[1] According to Roessler *et al.* (1978). P = probability of chance decision.

### 5.1.4 Susceptibility of raw milk to induced hydrolytic rancidity

The susceptibility of milks to induced rancidity has been illustrated in Fig. 5.1. It is evident that the initial ADV's were not affected by the level of "Protec" supplementation ($p>0.05$). However, ADV's for all milks increased several-fold after whipping and 15, 30 and 60 min incubation at 37°C . Raw milk had an ADV of 5.3 when 0% PLFS milk was incubated for 15 min, but higher levels of supplementation of "Protec" resulted in smaller increases in ADV's. At 30 or 60 min incubation time, the increase in free fatty acid content of the milks was less pronounced with increased levels of PLFS in the diet (Appendix F). This suggests a slight lowering of the susceptibility of milks to hydrolytic rancidity at the higher levels of "Protec" incorporation.

### 5.1.5 Susceptibility of milk to induced oxidative changes

"Protec" did not appear to affect the susceptibility of freshly homogenized and pasteurized milk to oxidative rancidity ($p>0.05$, Table 5.6). The addition of 1 ppm Cu to milk uniformly increased the TBA values. However, there was no significant difference between the TBA values of Cu-treated milks with various levels of PLFS supplementation. Fluorescent light treatment of milks for 3 and 5 days respectively, resulted in the development of slight off-flavours as detected by experienced dairy

Table 5.6 Susceptibility of milks to oxidative rancidity[1]

| Sample/Treatment | % Protec 0 | 3 | 6 | 9 | SE | $r^2$ |
|---|---|---|---|---|---|---|
| | ----------TBA---------- | | | | | |
| Initial | 0.026 | 0.022 | 0.022 | 0.024 | 0.000 | -0.12 |
| Copper treatment | 0.047 | 0.049 | 0.056 | 0.043 | 0.003 | -0.02 |
| Fluorescent light: | | | | | | |
| 3 days | 0.021 | 0.012 | 0.023 | 0.018 | 0.002 | 0.02 |
| 5 days | 0.022 | 0.014 | 0.022 | 0.018 | 0.004 | -0.06 |

[1]Means within the same row followed by the same or no superscript are not significantly different ($p > 0.05$).

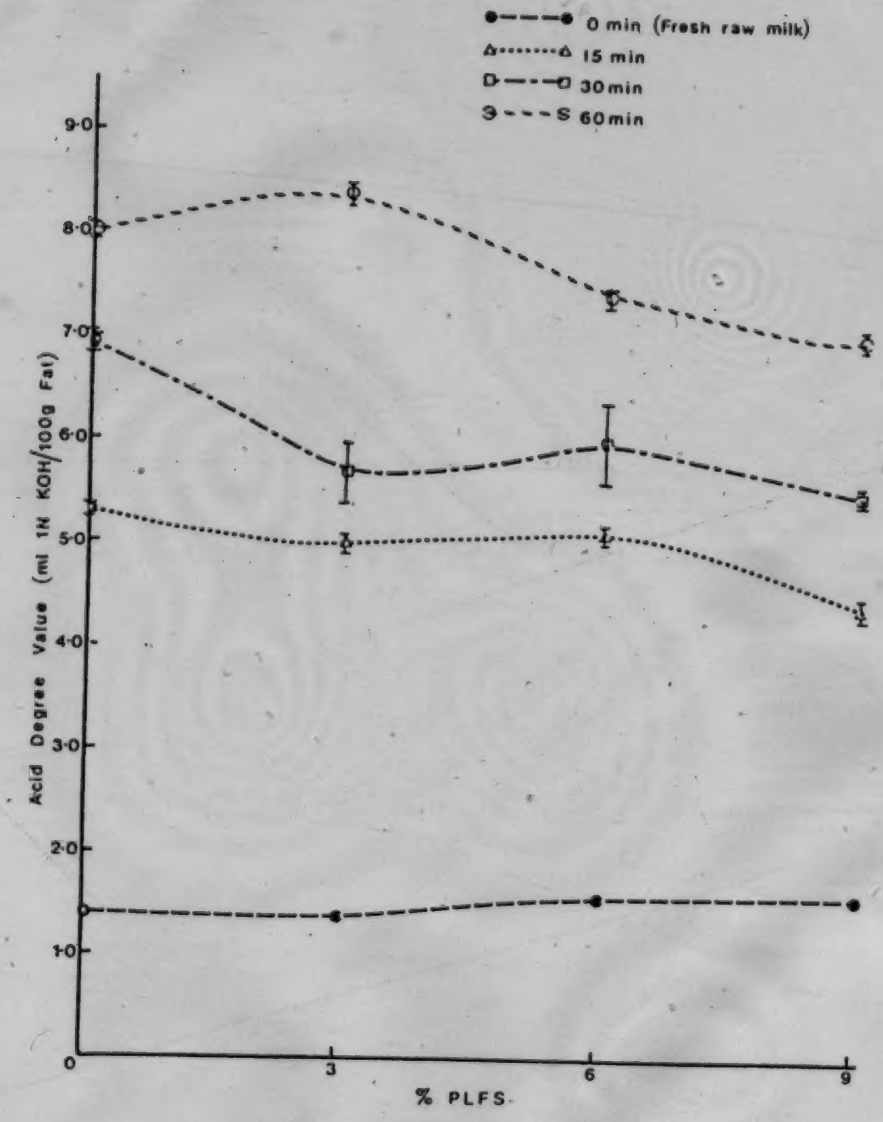



Fig. 5.1 Susceptibility Of Raw Milk To Induced Hydrolytic Rancidity

tasters. TBA values were low with no consistent pattern.

### 5.1.6 Effect of "Protec" on butter characteristics

Variation in absolute data for butter characteristics were often the result of animal and period differences (Appendix G). Consequently, data concerning the effect of "Protec" on the butter characteristics of the individual pairs of cows have been included in this section.

#### 5.1.6.1 Sensory characteristics of butter

Only one triangle test was carried out on butter as a preliminary test in preparation for sensory analysis of butter in Experiment II.

When presented with samples in a triangle test, the untrained panel was able to detect a slight difference in butter softness at the 6% level of "Protec" supplementation (Table 5.7). The probability of panelists making the correct decision based on chance was shown to be lowest for the 0% vs 6% PLFS butters, followed by the 0 vs 9% PLFS and the 0% vs 3% PLFS butters.

All butters were judged by trained dairy tasters to be of acceptable quality before and after 28 days of storage at 4°C.

Butter graders awarded a grade of 39 out of the customary 41 points for each of the fresh butter samples.

Table 5.7 Expt. I Panelists Correctly Identifying Odd Butter Sample in Triangle Test and Associated Levels of Significance

| Comparison | Panelists correctly identifying odd sample | Probability[1] | Degree of difference | Type of difference |
|---|---|---|---|---|
| O vs 3% PLFS | 3/18 | 0.967 | - | - |
| O vs 6% PLFS | 10/18 | 0.043 | Slight | Softness |
| O vs 9% PLFS | 8/18 | 0.223 | - | - |

[1] According to Roessler *et al.* (1978). (Probability of chance decision)

Table 5.8 Effect of "Protec" on butters obtained from milks of individual pairs of cows

| Butter Characteristic | Pair | 0 | 3 | 6 | 9 |
|---|---|---|---|---|---|
| Peroxide value[1] (meq/kg) | 1 | 0.199 | < 0.007 | < 0.007 | 0.155 |
| | 2 | 0.069 | 0.199 | < 0.007 | < 0.007 |
| | 3 | < 0.007 | 0.086 | 0.200 | < 0.007 |
| | 4 | < 0.007 | < 0.007 | 0.078 | 0.198 |
| Oiling-off (w/w %) | 1 | 2.0 | 3.2 | 2.5 | 2.2 |
| | 2 | 1.8 | 1.8 | - | 2.1 |
| | 3 | 2.2 | 2.6 | 2.9 | 1.9 |
| | 4 | 0.9 | 1.5 | 1.8 | 1.9 |
| | Mean | 1.7 | 2.3 | 2.4 | 2.0 |
| | SD | 0.50 | 0.67 | 0.45 | 0.13 |
| | Coefficient of variation (%) | 29 | 29 | 19 | 6.5 |
| Softening point ($^{o}$C) | 1 | 33.1 | 32.3 | 31.8 | 32.7 |
| | 2 | 30.8 | 32.3 | 31.0 | 31.7 |
| | 3 | 34.3 | 31.9 | 32.5 | 31.4 |
| | 4 | 32.7 | 34.5 | 33.0 | 32.9 |
| | Mean | 32.7 | 32.8 | 32.1 | 32.2 |
| | SD | 1.2 | 1.0 | 0.8 | 0.6 |
| | Coefficient of variation (%) | 3.7 | 3.0 | 2.5 | 1.9 |
| Dropping point ($^{o}$C) | 1 | 33.8 | 33.0 | 31.4 | 33.3 |
| | 2 | 33.6 | 32.8 | 30.4 | 31.4 |
| | 3 | 33.8 | 33.0 | - | 32.4 |
| | 4 | 34.3 | 34.0 | 34.0 | - |
| | Mean | 33.9 | 33.2 | 31.9 | 32.4 |
| | SD | 0.2 | 0.5 | 1.5 | 2.5 |
| | Coefficient of variation | 0.6 | 1.5 | 4.7 | 2.5 |

[1] Peroxide value of < 0.50 meq/kg is considered of acceptable quality (Buchanan and Rogers, 1973)

### 5.1.6.2 Oxidative stability of butters

Peroxide values for the fresh butters were consistently low (Table 5.8). In all cases values were less than 0.50 meq/kg, a value which has been reported to represent significant oxidation occurring in butter after prolonged storage (Buchanan and Rogers, 1973).

### 5.1.6.3 Level of unsaturation of butters

The level of unsaturation as determined by the iodine value (IV) increased in all cases at the 9% level of "Protec" supplementation over the control (0%) level (Fig. 5.2). For pair 1, a linear increase was observed between the amount of "Protec" fed and the iodine value of the resulting butters. The iodine values ranged from 40 for the control butter, to 47 when 9% "Protec" was fed. Smaller increases were noted in the case of the second pair, where the initial iodine value of 43 decreased slightly at the 3% level of supplementation, then increased at the 9% level to an IV of 45. Pair 3 had a similar pattern, however the initial IV (47) was higher than that of the other pairs. A fair increase in the iodine value was noted for pair 4, where the initial IV of 39 increased to 46 at the 9% level of supplementation. A small decrease occurred at the 6% level, however this value (40) was still slightly higher than the initial IV.

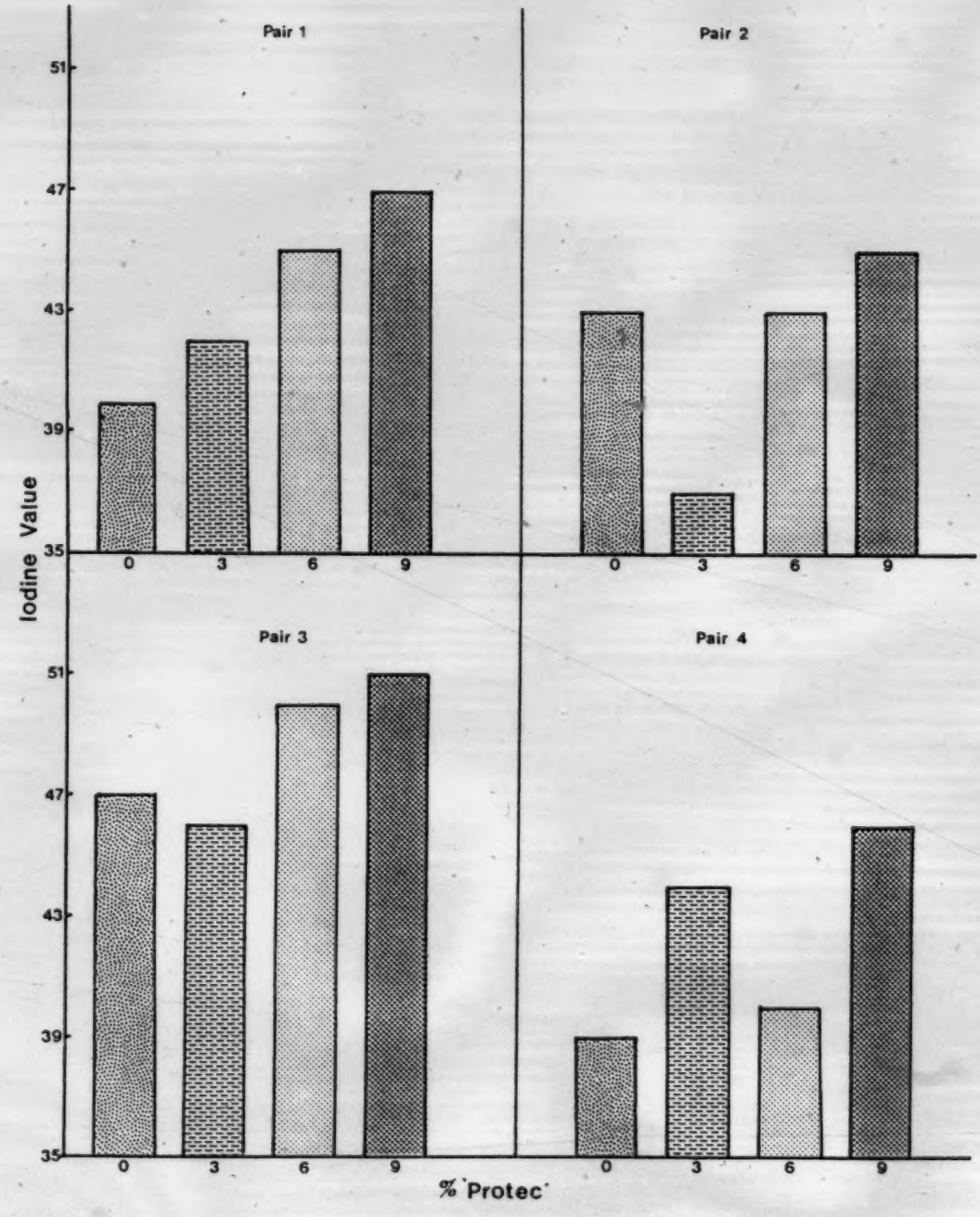



Fig. 5.2 Effect of "Protec" on the level of unsaturation of butters

Although fluctuations occurred in the level of unsaturation due to individual cow differences, there was a significant correlation ($p<0.05$) between the level of "Protec" in the diets and the degree of unsaturation, as indicated in a Pearson's Correlation analysis (Table 5.9).

**5.1.6.4 Hardness of butter**

Penetrometry studies were carried out to determine the effect of "Protec" in the diets of cows on the hardness of resulting butters. An excellent correlation ($p<0.01$) was observed between the iodine values and the hardness of the butters obtained (Table 5.9).

The general trend was a decrease in hardness with increased levels of supplementation (Fig. 5.3). In the case of pair 1, butter hardness decreased from 2.07 to 0.75 $kg/cm^2$ as the level of "Protec" increased from 0 to 9%. Butters obtained from pair 2 were slightly harder at the 3% level of supplementation than at the 0% level, however, butters from the 6 and 9% diets became progressively softer. Butter hardness was substantially lower for pair 3 cows than for other pairs of cows fed the various diets. Penetrometry measurements for pair 3 decreased to 0.45 $kg/cm^2$ at the 6% level and increased slightly when 9% "Protec" was fed. Butter from pair 4 cows decreased in hardness when "Protec" incorporation rose from 0 to 6%, then increased slightly when 9% "Protec"was fed.

Table 5.9 Inter-relationships among butter characteristics[1] as depicted by Pearson's Correlation Coefficients[2]

| | Level of "Protec" in diets | Iodine Value | Hardness | Softening point | Dropping point | Oiling-off |
|---|---|---|---|---|---|---|
| Iodine Value | 0.5144<br>n=16<br>p=0.042 | | | | | |
| Hardness | -0.6026<br>n=16<br>p=0.013 | -0.8058<br>n=16<br>p=0.000 | | | | |
| Softening point | -0.2574<br>n=16<br>p=0.336 | -0.0621<br>n=16<br>p=0.819 | 0.1195<br>n=16<br>p=0.659 | | | |
| Dropping point | -0.5859<br>n=14<br>p=0.028 | -0.2615<br>n=14<br>p=0.367 | 0.4518<br>n=14<br>p=0.105 | 0.6381<br>n=14<br>p=0.014 | | |
| Oiling-off | -0.2056<br>n=16<br>p=0.445 | 0.3424<br>n=16<br>p=0.194 | -0.4304<br>n=16<br>p=0.096 | -0.4372<br>n=16<br>p=0.090 | -0.4698<br>n=13<br>p=0.105 | |
| Solid Fat ($25^{0}C$) | -0.5358<br>n=13<br>p=0.059 | -0.5779<br>n=13<br>p=0.039 | 0.6027<br>n=13<br>p=0.029 | 0.3383<br>n=13<br>p=0.258 | 0.8589<br>n=13<br>p=0.000 | -0.6269<br>n=13<br>p=0.022 |

[1] Latin Square Design used

[2] $p<0.05$ is significant at the 95% confidence level; $p<0.01$ is significant at the 99% confidence level.

[3] The three entries for each correlation are, resp., the correlation coefficient, the number of cases analyzed, and the level of significance.

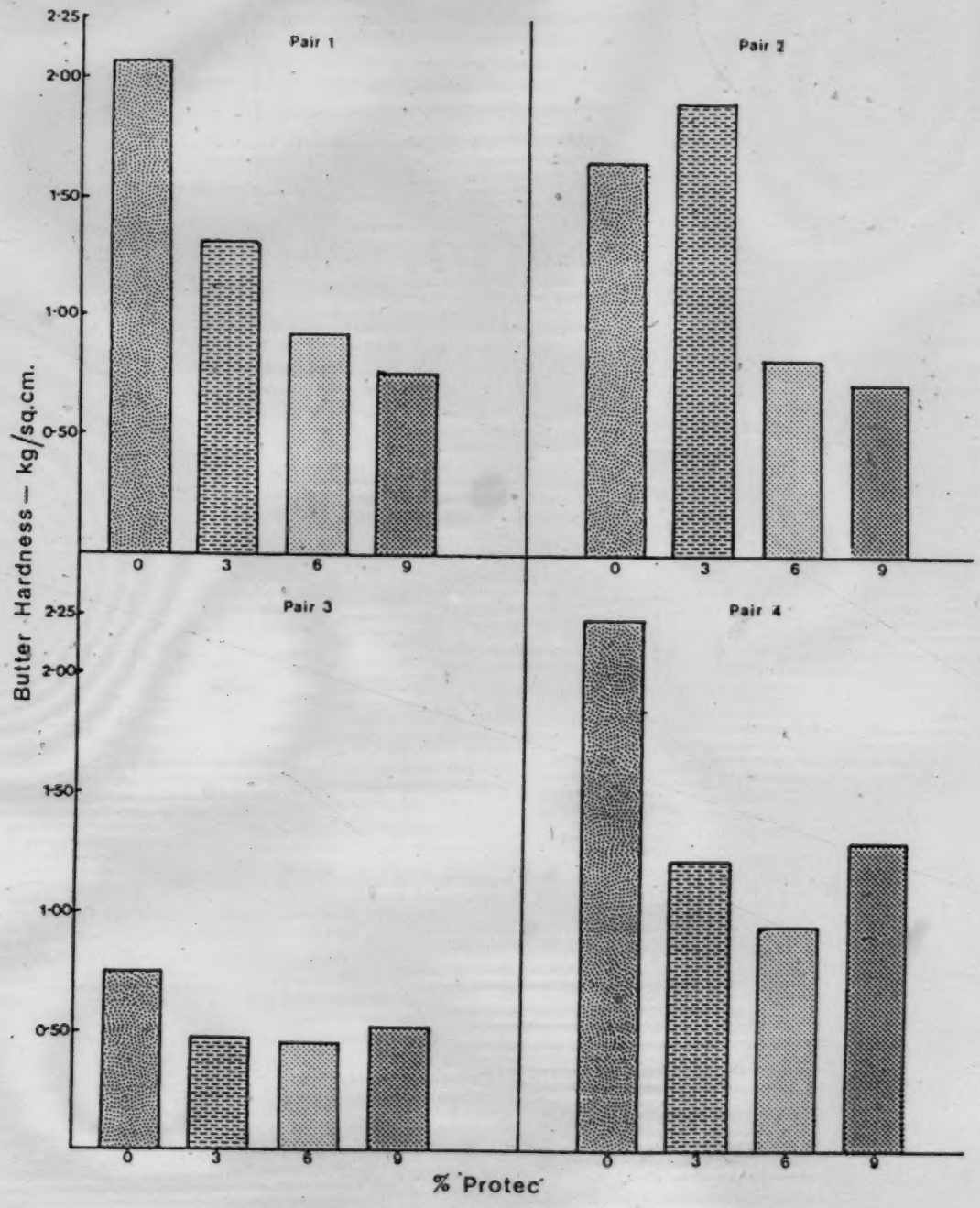



Fig. 5.3 Effect of "Protec" on butter hardness

Despite differences between pairs of cows, a correlation analysis illustrated that there was a significant correlation between the level of "Protec" in the diets and the resulting hardness of the butters ($p = 0.013$, Table 5.9).

### 5.1.6.5 Oiling-off of butter

As shown in Table 5.8, the amount of oiling-off (w/w%) of butters generally increased to the 6% level, after which there was a slight decline. In most cases however, the amount of free oil at 9% supplementation was greater than at the 0% level. Control butters (0% "Protec") from pairs 1, 2, and 3 displayed similar oiling-off patterns, while that of pair 4 was substantially lower.

### 5.1.6.6 Softening point of butter

Although some trends in the softening point (SP) of butters were observed, statistical analysis of the data indicated that there was no significant correlation between "Protec" level and the softening point (Table 5.8). In the case of pair 1, the SP decreased progressively to the 6% level of "Protec" supplementation, then increased at the 9% level. The softening point of butter obtained from pair 2 increased from 30.8°C to 32.3°C as the amount of "Protec" in the diet increased from 0 to 3%, then decreased to 31.0°C at the 6% level followed by a slight increase at the 9% level. For pair 3 a higher SP was observed at 0% "Protec" as compared with other butters from the same diet. A depression

in SP was noted at the 3% "Protec" level followed by an increase, while the SP at 9% was lowest for this pair of cows. The softening point of butter obtained from pair 4 was elevated from 32.7°C at the 0% level to 34.5°C at 3% "Protec", after which it decreased to 33.0°C and 32.9°C at the 6 and 9% "Protec" levels respectively.

**5.1.6.7 Dropping point of butter**

The dropping point of butters correlated well ($p = 0.014$) with softening points recorded (Table 5.9). However, although no correlation was observed between the softening point of butters and "Protec" supplementation, a significant correlation was recorded between the dropping point and the level of "Protec" in the diets. The reduced number of samples analyzed for dropping point (14 vs 16 for softening point) could possibly account for the increased level of significance noted between the dropping point and "Protec" supplementation. In general, the dropping point decreased linearly with increase in "Protec" supplementation (Table 5.8). Only small variations were observed in the butters from pairs of cows fed each of the four diets.

The dropping point of butters from pair 1 decreased to 31.4°C at the 6% level, and increased at the 9% level to 33.3°C. At 9% the dropping point was however slightly less than that at 0% "Protec". A similar pattern occurred with butters obtained from pair 2. In the case of butters from pair 3, the dropping point decreased from 33.8°C at 0%

"Protec" to 32.4°C at 9% "Protec". The dropping point of butters from pair 4 was fairly constant, (34.3°C at 0% "Protec" to 34.0°C at the 6% level of "Protec" supplementation).

**5.1.6.8 Solid fat content of butters at different temperatures**

As anticipated, the percentage of solid fat in each of the experimental butters decreased with increase in temperature (Table 5.10). Butters from cows fed "Protec" tended to contain less solid fat at each temperature than butter from cows not fed "Protec". However, this trend was linear only at 25°C (Appendix G, $p<0.05$). Pair 4 cows produced butter of slightly higher solid fat content than the other cows even when no "Protec" was incorporated in the diets. With 3% and 6% "Protec", the general trend was a decrease in the proportion of solid fat, although at 6% this parameter was slightly higher than that at 3%. A complementary pattern was observed with the IV of pair 4, where the solid fat content increased with "Protec" supplementation, but decreased slightly at the 6% level.

For the other pairs of cows (1, 2, 3) the solid fat content of butters appeared to be the lowest when fed diets containing 6% "Protec".

A Pearson's Correlation analysis (Table 5.9) indicated that the solid fat content of butters at 25 °C was related to several other parameters. Similar correlations were also

Table 5.10 Solid Fat Content of Butters

| Characteristic | | Pair | % Protec 0 | 3 | 6 | 9 |
|---|---|---|---|---|---|---|
| Solid fat (%) | 0°C | 1 | 45.53 | 45.28 | 40.60 | 41.50 |
| | | 2 | 44.85 | 46.73 | 28.30 | 38.59 |
| | | 3 | 39.15 | 40.98 | - | 36.17 |
| | | 4 | 50.36 | 45.05 | 45.89 | - |
| | | Mean | 44.97 | 44.51 | 38.26 | 38.75 |
| | | SD | 3.98 | 2.14 | 7.37 | 2.18 |
| | | Coefficient of variation (%) | 8.85 | 4.81 | 19.26 | 5.62 |
| | 5°C | 1 | 42.26 | 40.29 | 36.98 | 36.68 |
| | | 2 | 39.38 | 43.43 | 25.95 | 33.71 |
| | | 3 | 36.08 | 36.90 | - | 33.37 |
| | | 4 | 45.07 | 40.35 | 41.32 | - |
| | | Mean | 40.70 | 40.24 | 34.75 | 34.59 |
| | | SD | 3.34 | 2.31 | 6.47 | 1.49 |
| | | Coefficient of variation (%) | 8.21 | 5.74 | 18.62 | 4.31 |
| | 10°C | 1 | 32.81 | 32.88 | 27.84 | 30.10 |
| | | 2 | 30.74 | 33.58 | 17.77 | 26.56 |
| | | 3 | 29.47 | 29.64 | - | 26.19 |
| | | 4 | 37.05 | 31.89 | 33.60 | - |
| | | Mean | 32.52 | 32.00 | 26.40 | 27.62 |
| | | SD | 2.87 | 1.49 | 6.54 | 1.76 |
| | | Coefficient of variation (%) | 8.82 | 4.66 | 24.77 | 6.4 |

Table 5.10 (Continued)

| Characteristic | Pair | % Protec 0 | 3 | 6 | 9 |
|---|---|---|---|---|---|
| 15°C | 1 | 25.15 | 23.28 | 21.41 | 23.80 |
| | 2 | 24.14 | 24.50 | 10.38 | 20.65 |
| | 3 | 23.43 | 23.62 | - | 22.06 |
| | 4 | 28.75 | 25.86 | 27.41 | - |
| | Mean | 25.37 | 24.32 | 19.73 | 22.17 |
| | SD | 2.05 | 1.00 | 7.05 | 1.29 |
| | Coefficient of variation (%) | 8.08 | 4.11 | 35.73 | 5.82 |
| 20°C | 1 | 17.49 | 14.40 | 13.17 | 15.43 |
| | 2 | 16.64 | 15.67 | 0.98 | 12.71 |
| | 3 | 15.00 | 15.47 | - | 14.10 |
| | 4 | 20.10 | 17.46 | 19.43 | - |
| | Mean | 17.31 | 15.75 | 11.19 | 14.08 |
| | SD | 1.84 | 1.10 | 7.66 | 1.11 |
| | Coefficient of variation (%) | 10.63 | 6.98 | 68.45 | 7.88 |
| 25°C | 1 | 13.39 | 10.93 | 9.72 | 11.62 |
| | 2 | 12.48 | 11.45 | - | 8.64 |
| | 3 | 11.43 | 11.33 | - | 10.29 |
| | 4 | 16.27 | 12.34 | 14.82 | - |
| | Mean | 13.39 | 11.51 | 12.27 | 10.18 |
| | SD | 1.80 | 0.52 | 2.55 | 1.22 |
| | Coefficient of variation (%) | 13.44 | 4.52 | 20.78 | 11.98 |

noted at other temperatures. For example, the iodine value correlated with the solid fat content of butters at 0, 5, and 25°C, indicating that the level of unsaturation dictated the amount of solid fat present at these temperatures. There was a definite correlation between the solid fat content at 0, 5, 10, & 25°C and the hardness of these butters at 10°C, thus suggesting that the nature of glycerides in the milk fat influences the hardness of butter. Also, a positive correlation was noted between the solid fat contents of butters at the higher temperatures (15, 20, 25°C) and the tendency for oiling to occur. The implications of this can be readily appreciated since oiling off is known to occur more readily at temperatures above 10°C particularly when polyunsaturated fatty acids are present (Kieseker and Eustace, 1975).

There was an excellent correlation between the solid fat content at all temperatures (0, 5, 10, 15, 20, 25°C) and the dropping point of the butters prepared, although this relationship was not apparent between the solid fat and the softening points. Again, the difference in the number of samples tested, or the slightly larger margin of error for softening point determination, could account for this discrepancy.

### 5.1.7 Discussion

The feed consumption pattern of Holstein dairy cows involved in this study was consistent with the results of previous experiments carried out using different oilseeds (Barbano and Sherbon, 1980; Grieve, 1976). This implies that no adverse effects with respect to feed intake result when up to 9% canola-based "Protec" was incorporated into diets. Replacement of part of the basal ration with "Protec" resulted in isonitrogenous diets of slightly increased energy content. This trend is consistent with that reported by Bines *et al.* (1978); Smith *et al.*, (1978); and Palmquist and Jenkins, (1980), for low levels of protected lipid supplementation.

No significant changes occurred in milk yield and composition when varying levels of "Protec" were fed. Several studies have reported that milk yield was not significantly affected by protected lipid supplement in the diet (Pan *et al.*, 1972; Plowman *et al.*, 1972; Goering *et al.*, 1977; and Smith *et al.*, 1978). On the other hand, it has been consistently demonstrated that the butterfat levels of milk resulting from PLFS incorporation are increased (Scott, *et al.*, 1970; Scott and Cook, 1973; Goering *et al.*, 1977).

The absence of such a response in this study may be due to one or more factors. The effect of pelletization of the feed may have decreased the level of protection somewhat (Fisher, 1975; Scott, 1975), hence the free fat resulting

could contribute to depression of intramammary synthesis of fatty acids (Scott, 1975; Storry & Brumby, 1979). The fact that the initial feeding regime of the cows (prior to being placed on test diets) entailed offering a rolled ration, could also account for the absence of an increase in the fat content, as an adjustment period of varying times may be needed (Parr, 1982). Also, diet manipulations due to previous experimental treatments may have affected the "adaptability" of the cows. Finally, since test animals involved were at various lactation periods (See Appendix A), the capacity for utilizing optimum genetic potential for milk production may have been reduced.

Most of the responses observed in this study, (eg. butter softening, level of unsaturation, fatty acid analyses) are typical of feeding PLFS.

Results of sensory analyses of milk suggest that there may be slight flavour differences in milks at the 6 and 9% level of supplementation. However, in addition to the presence of "Protec", milk collection procedures, and perhaps stage of lactation, could also be possible reasons for flavour differences. Thus firm conclusions regarding the effect of graded levels of "Protec" on milk flavour can be tentative at best.

Sensory tests on butter indicate that there is a slight softening effect at the 6% level of "Protec". A similar trend has been documented by several researchers, when butters containing high levels of unsaturated fatty acids

were produced by various techniques (Alsafar, 1974; Kieseker & Eustace, 1975; Wood *et al.*, 1975).

ADV's of "whipped" milks indicated the onset of rancidity and were typical of activated milks (Thomas *et al.*, 1955; Kitchen & Aston, 1970). Milks were shown to be slightly less susceptible to induced hydrolytic rancidity at the 6% and 9% levels of "Protec" in the diets. A similar trend was reported by Astrup (1980), when it was shown that rancid flavour was inhibited in milks obtained when protected canola oil was fed.

Initial TBA values of milks were typical of 'fresh' homogenized and pasteurized milk (King, 1962). There appeared to be no significant difference in the susceptibility of milks to copper-induced oxidation. This is contrary to previous reported studies in which high levels of supplementation of safflower oil were used and it was found that resulting milks tended to have a slight oxidized flavour (Edmondson, *et al.*1974; Goering *et al.*, 1976). This discrepancy could be due to a reduced effect at lower levels of "Protec", and/or improved oxidative stability obtained when protected oilseeds rather than oils are fed (Haase, 1977). In addition, the difference in the fatty acid composition of canola oil (high in C18:1) and safflower oil (high in C18:2), may account for results obtained in this work. In the fluorescent light study, the milks did not become oxidized as shown by TBA values, even after 5 days illumination. Air has been shown to be required for

off-flavours (lipid oxidation) to occur in milk (Gregory *et al.*, 1972). Hence, the absence of a response could probably be explained by the fact that flasks used were sealed with Saran Wrap, thus limiting the air available to the milk system.

The oxidative stability of butters was good, peroxide values being typical of fresh butters (Buchanan & Rogers, 1973). Due to the minimal amounts of hydroperoxides present in some butters, difficulty was experienced in the end-point determination of the reaction (Gray, 1978). Although there were some variations in the peroxide values, supplementation with "Protec" had no obvious effect.

One of the major effects of "Protec" supplementation is the increase in C18:2 levels (Kreula & Norlund, 1974; Wood *et al.*, 1975; Barbano & Sherbon, 1980). This effect was also displayed in this study, using low levels of "Protec" supplementation. As illustrated by fatty acid analyses (Sporns *et al.*, unpublished data; Jelen *et al.*, 1982), the fatty acid composition of milk fat obtained with the 6% and 9% diets was significantly different from that of the control and 3% "Protec" diets. In general, an increase in the C18 fatty acids occurred at the expense of those with the lower molecular weight chains. This pattern has also been noted by Goering *et al.* (1976), Astrup *et al.* (1979), Barbano and Sherbon (1980), and is consistent with the levels of unsaturation noted. Feeding ground canola seeds to lactating Holsteins resulted in similar trends in fatty acid

composition (Kennelly and Fenton, 1982), suggesting either that the "Protec" used in this experiment was not fully protected, or that the canola seed itself exhibits a natural "protection".

In all cases, there was an increase in iodine value when 6% and 9% "Protec" was fed, suggesting an increase in unsaturation. The general trend for hardness of butters to decrease with supplementation is consistent with the concurrent increase in unsaturation. More noticeable decreases in butter hardness appeared at the 6% level of "Protec" in the diets, afterwhich the decrease was less pronounced.

Oiling off of butters tended to increase with increase in supplementation. As appropriately hypothesized by Wood *et al.* (1975), at 20°C and with fairly high concentrations of linoleic acid, there is insufficient surface area of fat crystals to retain the higher proportion of liquid fat in the butter, hence the occurrence of oiling off. It should be noted here that the high standard deviation among triplicate samples was due to the high potential error in this determination (Mortensen and Danmark, 1980). The slight decrease in oiling-off which occurred at the 9% level of supplementation (with respect to the 6% level) was consistent with other data reported (eg. dropping point and solid fat data). The oiling-off tendency observed for the more "unsaturated butters" is in accordance with data reported by Eustace, (1975); Alsafar, (1974), and is due to

the proportion of liquid fat present.

Dropping point and softening point data which essentially both provide an indication of the melting characteristics of butter, were in the normal range as reported by Parodi & Dunstan, (1971). Dropping point (DP) results more clearly illustrated that there was a decrease to the 6% level, afterwhich (i.e at 9%) there was a slight increase. However, the DP at 9% was still lower than that at 0% "Protec". The general decrease in the dropping and softening point with increased supplementation was in accordance with the increase in unsaturation, and has been previously reported (Buchanan and Rogers, 1973).

These results were complemented by the solid fat data at the different temperatures. Again, the general trend was a decrease in the solid fat content to the 6% level, with a small increase at the 9% level of "Protec" in the diet. The unexpected increase in the dropping point and percent solid fat at 9% supplementation could be accounted for if there was a changed pattern in the concentration of high molecular weight glycerides at this level of "Protec" incorporation.

## 5.2 Experiment II -- Storage stability of "Protec" and the effect of feeding "Stored Protec" on milk and butter quality

The susceptibility of the canola-based "Protec" itself to oxidative changes during controlled storage was determined prior to feeding 6% of the oxidized feed in this experiment.

### 5.2.1 Susceptibility of "Protec" to oxidative changes during storage

The extent of oxidation as depicted by peroxide values was the greatest for "Protec" that had been stored at 40°C and exposed to air (Table 5.11). The peroxide value of the frozen control "Protec" remained low (1.5 to 1.8 meq/kg) throughout the twelve week storage period, while samples stored under other conditions (4°C, room temperature, and 40°C) displayed a rise in peroxide value with time.

### 5.2.2 Effect of "Stored Protec" on feed consumption, milk yield , milk composition and quality of milk and butter

Since results of Experiment I suggest 6% as a possible threshold level for addition of "Protec" to diets, this level was used to investigate the effects of feeding the stored product to lactating dairy cows in the second experiment.

Table 5.11 Storage Stability of "Protec"

| Storage conditions | Storage time (months) | | |
|---|---|---|---|
| | 1 | 2 | 3 |
| | ----- Peroxide value (meq/kg)[1] ------ | | |
| Frozen (-25°C) | 1.51 | 1.73 | 1.77 |
| Refrigerated (4°C) | - | 4.46 | 8.07 |
| Held at room temperature | 2.89 | 6.52 | 9.47 |
| Incubated (40°C) | | | |
| - closed container | - | - | 15.33 |
| - open container | - | 17.25 | 24.65 |

[1]Initial peroxide value = 1.40 meq/kg.

### 5.2.2.1 Feed Consumption

There was no significant difference in feed intake when diets containing no "Protec", 6% fresh "Protec", and 6% "Stored Protec", were offered to lactating dairy cows in a random block design ($p>0.05$, Table 5.12). Intakes of hay increased slightly with diets containing "Protec", however, the standard deviations were quite high, probably due to variation in consumption of cows on the same diet. A similar trend was noted for grain intake, where it ranged from 10.7 kg/day for diet containing no "Protec" to 11.2 kg/day for diet containing 6% "Stored Protec".

### 5.2.2.2 Milk yield and composition

Cows being fed diets containing no "Protec" and 6% "Protec" produced similar amounts of milk during experimental trial (Table 5.12). Although milk yield and FCM yield resulting from 6% "Stored Protec" appeared slightly higher, these differences were not significant ($p>0.05$). Similarly, diet had no apparent effect on level of butterfat, protein, lactose, total solids and solids-non-fat of milk collected from individual cows (Table 5.12) or of the milk composited from cows fed the same diet (Table 5.13). There was no significant difference between butterfat content as determined by the Milkoscan Infra-Red method and the Mojonnier method ($p>0.05$). Differences in butterfat content were small and not significant ($p>0.05$). The protein content was similar for all milks, although there was a

Table 5.12 Effect[1] of Fresh and Stored "Protec" on Feed Consumption, Milk Yield and Milk Composition of Lactating Dairy Cows (Average Data for 4 Cows Per Diet)

| | Without Protec | Protec | | SE |
|---|---|---|---|---|
| | | Fresh | Stored | |
| Feed Consumption (kg/day) | | | | |
| Hay | 6.9 ± 0.9 | 7.4 ± 0.4 | 8.0 ± 1.2 | 0.25 |
| Grain | 10.7 ± 0.9 | 10.7 ± 0.9 | 11.2 ± 0.9 | 0.27 |
| Milk Yield (kg/day) | | | | |
| Milk | 23.8 ± 2.4 | 23.8 ± 3.6 | 25.0 ± 1.2 | 0.76 |
| FCM (4% BF)[2] | 18.9 ± 3.2 | 17.9 ± 3.6 | 19.1 ± 3.1 | 0.95 |
| Butterfat[2] | 0.6 ± 0.2 | 0.6 ± 0.2 | 0.6 ± 0.2 | 0.05 |
| Protein | 0.8 ± 0.1 | 0.8 ± 0.1 | 0.8 ± 0.1 | 0.02 |
| Milk Composition (%) | | | | |
| Butterfat - Milkoscan | 2.5 ± 0.7 | 2.3 ± 0.7 | 2.4 ± 0.8 | 0.21 |
| - Mojonnier | 2.6 ± 0.8 | 2.4 ± 0.7 | 2.4 ± 0.7 | 0.21 |
| Protein | 3.4 ± 0.2$^{ab}$ | 3.5 ± 0.1$^{a}$ | 3.2 ± 0.2$^{b}$ | 0.04 |
| Lactose | 4.9 ± 0.2 | 4.9 ± 0.1 | 4.9 ± 0.1 | 0.04 |
| Total solids | 11.7 ± 0.7 | 11.5 ± 0.3 | 11.3 ± 0.3 | 0.19 |
| Solids-not-fat | 9.2 ± 0.3 | 9.2 ± 0.2 | 8.9 ± 0.3 | 0.07 |

[1]Mean ± SD (n = 4); Means within the same row followed by the same or no superscript are not significantly different ($p > 0.05$).

[2]Based on butterfat determined by Mojonnier procedure.

Table 5.13 Effect of Fresh and Stored "Protec" on the Composition of Milk and Butter Used for Quality Studies

| | Without Protec | Protec | |
|---|---|---|---|
| | | Fresh | Stored |
| Milk | | | |
| Butterfat - Milkoscan | 2.45 | 2.28 | 2.37 |
| - Mojonnier | 2.55 | 2.39 | 2.45 |
| Protein | 3.41 | 3.48 | 3.22 |
| Lactose | 4.92 | 4.93 | 4.88 |
| Total solids | 11.65 | 11.59 | 11.31 |
| Solids-non-fat | 9.20 | 9.31 | 8.94 |
| Butter | | | |
| Moisture (%) | 18.7 | 18.9 | 18.0 |
| Fat (%) | 74.8 | 76.6 | 75.7 |

slight decrease in this component when 6% "Stored Protec" was fed, compared to when 6% fresh "Protec" was fed ($p>0.05$). The lactose content of all milks concerned were the same (4.9%). Differences occurring in the total solids and in the solids-non-fat content were not statistically significant ($p>0.05$). The moisture and fat content of the respective butters produced from the milks were also similar ($p>0.05$).

**5.2.2.3 Sensory evaluation of milk and butter**

Homogenized and pasteurized milks were found to be of acceptable quality before and after controlled storage at 4°C. Untrained panelists were unable to distinguish between the milks produced from cows fed either of the "Protec" diets ($p>0.05$, Table 5.14). Only ten panelists (out of thirty) identified the correct sample when presented with milks obtained from 0% vs 6% fresh "Protec", and 0% vs 6% "Stored Protec", whereas only 9 (out of thirty) were able to distinguish between milk obtained from 6% fresh vs that from 6% "Stored Protec". Probability values indicate that statistically, the panels were unable to distinguish between milks when 6% fresh "Protec" and 6% "Stored Protec" was fed.

The same sensory panel was able to distinguish between butter from cows fed "Protec" and butter from cows not fed "Protec" ($p<0.05$, Table 5.15). Also, panelists were able to distinguish between butters from cows fed 6% fresh "Protec" and 6% "Stored Protec". In most cases, the spreadability was

Table 5.14 Expt. II: Panelists correctly identifying odd milk sample in triangle test and probability levels of significance

| Comparison | Panelists correctly identifying odd sample | Probability |
|---|---|---|
| 0 vs 6% "fresh Protec" | 10/30 | 0.568 |
| 0 vs 6% "stored Protec" | 10/30 | 0.568 |
| Fresh vs stored "Protec" | 9/30 | 0.714 |

recorded as the parameter contributing to the difference observed. When these butters were whipped, the panel could not distinguish between the flavour of butters for 0 vs 6% fresh "Protec" and 6% fresh "Protec" vs 6% "Stored Protec" (p=0.50, and 0.55 resp., Table 5.16), indicating that there was no real difference in the flavour of the butters made.

All butters prepared were judged by Federal Butter Graders to be of acceptable quality. A score of 39 points out of a total of 41 was awarded to each of these experimental butters.

**5.2.2.4 Susceptibility of raw milk to induced hydrolytic rancidity**

There was a slight increase in the Acid Degree Values of the initial raw milks when either fresh or stored "Protec" was fed to dairy cows (Table 5.17). Milk resulting from 6% "Stored Protec" had an ADV (1.25) similar to that resulting from 6% fresh "Protec" (1.39). Although ADV's of whipped and incubated milks increased several-fold over initial values, only small differences occurred between the ADV's of these raw milks at 30 min. incubation when 0% "Protec", 6% fresh "Protec", and 6% "Stored Protec" were fed.

**5.2.2.5 Susceptibility of homogenized and pasteurized milk to induced oxidative changes**

Table 5.15 Expt. II: Panelists correctly identifying odd butter sample in triangle test and probability levels of significance

| Comparison | Panelists correctly identifying odd sample | Probability | Degree of difference | Type of difference | |
|---|---|---|---|---|---|
| | | | | Flavor | Spreadability |
| 0 vs 6% fresh "Protec" | 16/24 | 0.001 | Slight - moderate | 3/16 | 16/16 |
| 0 vs 6% stored "Protec" | 15/24 | 0.003 | Slight - moderate | 8/15 | 13/15 |
| Fresh vs stored "Protec" | 15/24 | 0.003 | Slight - moderate | 10/15 | 13/15 |

Table 5.16 Experiment II: Comparison of butter flavour using the Signal Detection Test.

| Treatment | R[1] |
|---|---|
| 0 vs 6% "Stored Protec" | 0.55 |
| Fresh vs "Stored Protec" | 0.50 |

[1]Probability of distinguishing between flavour of two butter samples. Possible differences in spreadability were eliminated by whipping for 5 min. in a Kitchen-Aid mixer.

Table 5.17 Expt II: Effect of Fresh and Stored "Protec" on the Susceptibility of Milk and Butter to Hydrolytic and Oxidative Rancidity

| | Without Protec | Protec | |
|---|---|---|---|
| | | Fresh | Stored |
| **Susceptibility of Raw Milk to Induced Hydrolytic Rancidity** | | | |
| ADV - initial | 0.89 | 1.39 | 1.25 |
| - 30 min | 4.90 | 4.95 | 5.36 |
| - % increase | 551 | 356 | 429 |
| **Susceptibility of Milk to Induced Oxidation** | | | |
| TBA - initial | 0.016 | 0.031 | 0.017 |
| - copper | 0.031 | 0.070 | 0.048 |
| **Susceptibility of Butter to Oxidation During Storage** | | | |
| Peroxide value[1] (meq/kg) | | | |
| - initial | <0.007 | 0.038 | 0.051 |
| - one month storage | <0.007 | 0.041 | 0.060 |

[1] Peroxide Value of < 0.50 meq/kg is considered to be of acceptable quality regarding oxidation (Buchanan and Rogers, 1973).

The level of oxidation as indicated by TBA values was increased when 6% fresh "Protec" was fed as compared to when diets containing no "Protec" were given (Table 5.17). TBA value for milk obtained from 6% "Stored Protec" was similar to that resulting when no "Protec" was fed ($p>0.05$). Addition of copper caused a similar trend in TBA values, where milks from 6% fresh "Protec" had a higher value (0.070) than milk from 0% "Protec" and 6% "Stored Protec" (0.031 and 0.048 resp.).

**5.2.2.6 Oxidative stability of butter**

Peroxide values of all the butters concerned were low (<<0.50 meq/kg) even after one month's refrigerated storage (Table 5.17). This was in agreement with the lack of oxidized flavour in the butters noted by the trained dairy tasters.

**5.2.2.7 Effect of fresh and "Stored Protec" on butter characteristics**

Supplementation of 6% fresh "Protec" resulted in increased unsaturation of butter produced as indicated in Table 5.18. The iodine value of butter from 6% "Stored Protec" was essentially the same as that obtained when no "Protec" was fed.

Penetrometry readings were the highest for butters resulting when a standard ration (0% "Protec") was fed to cows. A decrease in the hardness of butters occurred when fresh "Protec" was incorporated in the diet, however, the

Table 5.18 Effect of Fresh and Stored "Protec" on butter characteristics

| Butter characteristics | Without "Protec" | "Protec" | |
|---|---|---|---|
| | | Fresh | Stored |
| Iodine value[1] | 28.3±1.1 | 31.5±0.2 | 27.8±0.8 |
| Hardness ($kg/cm^2$) | 1.05±0.02 | 0.64±0.06 | 0.98±0.04 |
| Oiling-off (w/w %) | 0.80±0.07 | 1.54±0.01 | 1.92±0.34 |
| Softening point ($^oC$) | 31.7±0.4 | 30.3±0.2 | 31.3±0.5 |
| Dropping point ($^oC$) | 31.8±0.1 | 30.4±0.0 | 31.0±0.1 |

[1]Mean ± Standard Deviation (n=4)

hardness of butters resulting from 6% "Stored Protec" (0.98 kg/cm²) was similar to that from 0% "Protec" (1.05 kg/cm²).

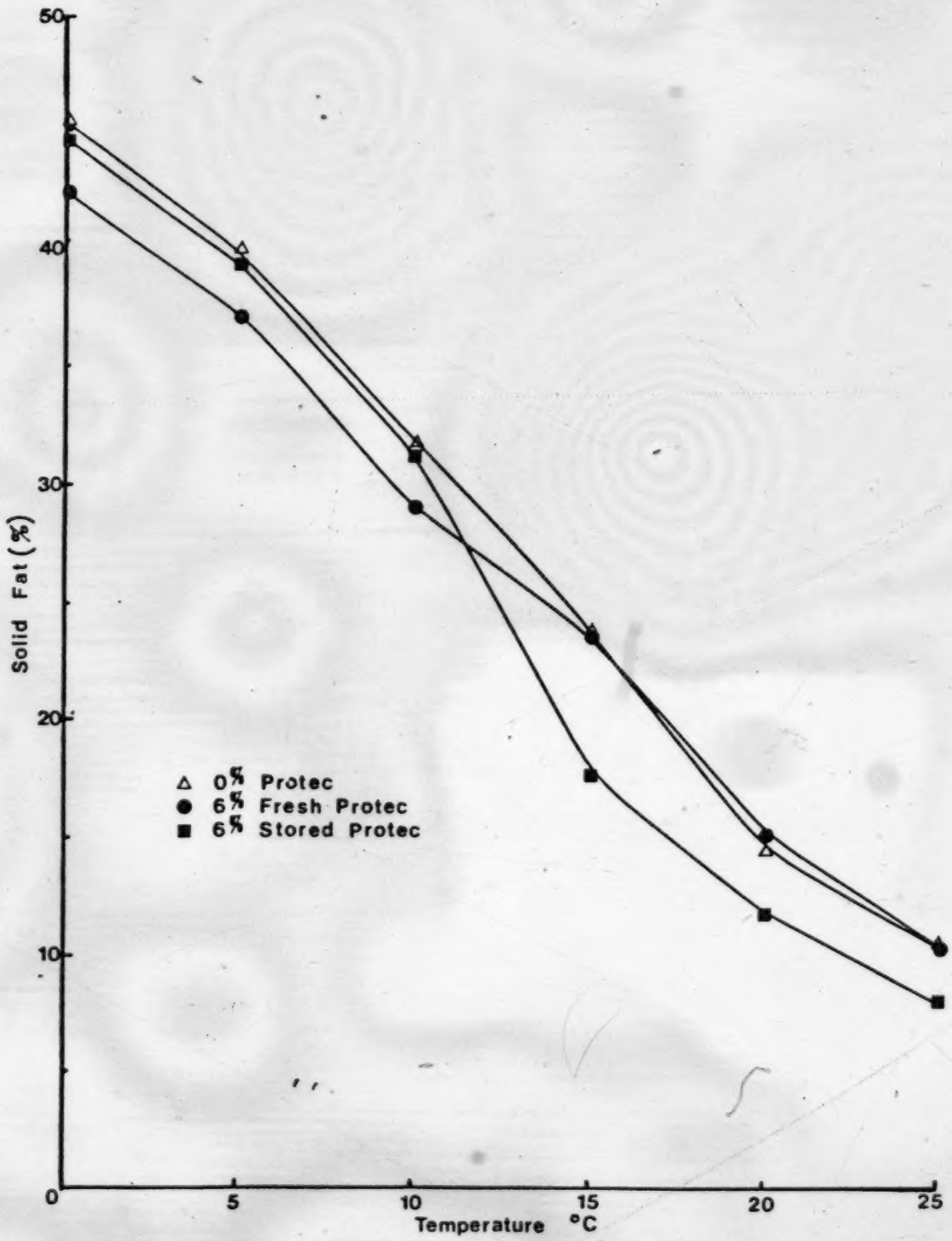
Small increases occurred in the oiling off of the butters prepared from milks when 0% "Protec", 6% fresh "Protec" and 6% "Stored Protec" were fed.

Softening point data of butter indicate a decrease with 6% fresh "Protec" in the diet. Similar softening points were attained with 0% "Protec" and 6% "Stored Protec".

Dropping point determinations produced a similar trend as noted for softening point. The lowest dropping point was observed with 6% fresh "Protec", while similar values were noted for butters from 6% "Stored Protec", and 0% "Protec".

Solid fat content decreased with increased temperature for all butters as expected (Fig. 5.4). Butter from cows fed 6% fresh "Protec" contained less solid fat at 0, 5, and 10°C than the control butter. At temperatures above 15°C, the "Stored Protec" butter contained less solid fat than the other two butters.

Fig. 5.4 Effect of fresh and stored 'Protec' on solid fat content of butter.



### 5.2.3 Discussion

Results of storage stability study indicated that "Protec" was most stable with respect to oxidation when stored under refrigeration conditions. The great increase in peroxide value that occurred at 40°C was indicative of fat oxidation, hence this product ("Stored Protec") was utilized to investigate whether intake by dairy cows would influence feed consumption, milk yield, composition and quality of milk and butter.

The intake of concentrate and hay was not affected by the inclusion of "Protec" in the diet or by the state of oxidation of this "Protec" ($p>0.05$, Appendix H). No negative effects on milk yield and composition of the resulting milks were noted when 0% "Protec", 6% fresh "Protec" and 6% "Stored Protec" were fed.

The quality of raw milks with respect to lipolytic rancidity was good, ADV's being typical of freshly drawn milk (Pillay *et al.*, 1980a). All milks when whipped and incubated for 30 min., became rancid. "Protec" however had no drastic effect on susceptibility of the milks to hydrolytic rancidity. The percentage increase in ADV was noted to be the lowest for milk when 6% fresh "Protec" was fed. This corroborated the results of Experiment I.

Milks resulting from diets containing no "Protec" and 6% "Stored Protec" had TBA values typical of good, unoxidized milk, whereas milk obtained when 6% fresh "Protec" was fed could be characterized as being slightly

oxidized (King, 1962). The increase in the TBA value with 6% fresh "Protec" included in the diet was in agreement with results reported by Goering *et al.*,1976, when a higher level of safflower- based PLFS was fed.

The absence of an increase in TBA values for milks produced with the 6% "Stored Protec" diet (even on addition of $Cu^{2+}$), could be due to several factors. For example, the formation of hydroperoxides, and subsequently carbonyl compounds (See Fig. 2.6) in the stored product, would reduce the level of unsaturation of the feed, hence a more saturated feed would be ingested. Milk resulting would then reflect this fatty acid pattern and thus contain less sites for oxidation to occur. Also, the bulk of evidence (Shipe, 1964 and Bruhn *et al.*, 1976), suggest that milk produced during early lactation may be more susceptible to oxidation. The cows being fed Diet 2 (fresh "Protec") were at a slightly earlier lactation than cows on diet 3 ("Stored Protec") (See Appendix A); this could possibly contribute to increased oxidation in milks from Diet 2. Finally, other factors arising from inherent differences between individual cows could account for increased TBA values for milks from Diet 2.

Results of sensory evaluation illustrated that there were no differences between milks when either fresh "Protec" or "Stored Protec" was fed. Similarly, panels could not distinguish between milk resulting from 6% fresh "Protec" and 6% "Stored Protec".

Butters resulting when "Protec" was fed were more spreadable and no undesirable flavours were detected. This was consistent with data from Experiment I and also with those previously reported. (Buchanan & Rogers, 1973; Kieseker, *et al.*, 1974). After one month's refrigerated storage, butters were judged to be of acceptable quality by trained panelists.

Objective measurements on butter were all complementary. The increased level of unsaturation observed with 6% fresh "Protec" was accompanied by a decrease in hardness as expected. Unsaturation and hardness values of butters resulting from "Stored Protec" were similar to those from 0% "Protec". This trend could be due to:

i. Disruption of double bonds in fatty acids of "Protec" during storage - by oxidation, thus decreasing unsaturation which is subsequently reflected in the milkfat

ii. Nature of characteristic fatty acids in butterfat obtained from individual cows' milk.

The low iodine values noted in general were possibly due to the later lactation period of cows in this trial. Oiling off increased with "Protec" supplementation, as also reported by Wood *et al.* (1975), when higher levels of PLFS were used.

Softening point and dropping point data complement the data indicating the degree of unsaturation. The lower melting range of butters at the 6% "Protec" level (as indicated by softening and dropping points) could therefore

be a result of higher unsaturation of these butters. The pattern is also seen in the solid fat analyses, where lower proportions of solid fat occurred for 0-10°C inclusively for 6% fresh "Protec". At higher temperatures, the abnormal behaviour of butters produced when 6% "Stored Protec" was supplemented in diets may be due to melting points of glycerides present.

## 5.3 Experiment III-- Evaluation of milk and butter from cows fed "Protec" in commercial dairy herds

The possibility of long-term effects of feeding commercially manufactured "Protec" was investigated in this experiment. Milk was obtained from local dairy herds (Holstein) which had been fed a "Protec" supplemented diet (5-10% according to farmers' indication) for at least three years. Composition and sensory quality of the milks and butters were evaluated. Control samples were obtained from two commercial herds which had never received any PLFS.

### 5.3.1 Composition of milk and butter

There was little difference in the composition of milk obtained from the two commercial dairy farms which had never included "Protec" in the feeding program and two that had been supplementing "Protec" for at least three years ($p>0.05$, Table 5.19). The average butterfat content of the milk produced from the "Protec"-containing diet was slightly

Table 5.19 Effect of "Protec" on the composition of milk and butter from commercial herds

| Component (%) | $C_1$ | $C_2$ | $C_{\bar{X}}$ | $P_1$ | $P_2$ | $P_{\bar{X}}$ |
|---|---|---|---|---|---|---|
| Milk | | | | | | |
| Butterfat | | | | | | |
| - Milkoscan | 3.79 | 3.62 | 3.70 | 3.79 | 3.75 | 3.77 |
| - Mojonnier | 3.74 | 3.58 | 3.66 | 3.78 | 3.74 | 3.76 |
| Protein | 3.10 | 3.14 | 3.12 | 3.07 | 3.24 | 3.16 |
| Lactose | 4.89 | 4.87 | 4.88 | 4.93 | 4.97 | 4.95 |
| Total solids | 12.41 | 12.26 | 12.34 | 12.50 | 12.63 | 12.56 |
| Solids-non-fat | 8.62 | 8.64 | 8.63 | 8.71 | 8.88 | 8.80 |
| Butter | | | | | | |
| Moisture | 16.4 | 15.4 | 15.9 | 14.2 | 15.3 | 14.8 |
| Fat | 79.6 | 81.3 | 80.4 | 83.8 | 82.8 | 83.3 |

higher than that of the control milk (no "Protec"), although this difference was not significant ($p>0.05$, Appendix I). Protein, lactose, total solids and solids-non-fat were similar for both control milks and milks resulting from "Protec" supplementation.

The butters prepared from milk produced at the four farms were of similar composition (Table 5.19). The overall moisture content of the control butters was slightly higher than that obtained when "Protec" was fed. The average fat content of the control butters was slightly lower than that of the "Protec"-butters.

**5.3.2 Flavour of milk and butter resulting from "Protec" supplementation**

All milks and butters were found by experienced dairy tasters to be free of flavour defects. The sensory panel used in the previous two experiments (Expt. I & II) was unable to distinguish between freshly homogenized, pasteurized milk obtained from "Protec"-fed herds and herds not being fed "Protec" ($p>0.05$, Table 5.20). Results of a replicate experiment using thawed samples that had been kept frozen at -40°C for 3 weeks were consistent.

Since it was illustrated in the previous experiments that softness of butter was amply described by penetrometry, the signal detection test was used to test only for possible flavour differences in whipped butters. The results of this test showed that there was no difference in the flavour of

Table 5.20 Expt. III: Panelists correctly identifying odd milk sample in triangle test and probability levels of significance

| Comparison | Panelists correctly identifying odd sample | Probability[1] |
|---|---|---|
| Control vs Protec-fed herds | | |
| Fresh | 7/14 | 0.149 |
| Frozen | 5/10 | 0.213 |

[1]According to Roessler *et al.* (1978).

the butters ($p>0.05$, Table 5.21).

### 5.3.3 Susceptibility of butter to oxidation

Peroxide values obtained for freshly made butters and one-month old butters were typical of good, unoxidized butter (Table 5.22). The average values for butters obtained when cows were fed a control diet and when "Protec" supplemented diet was fed were similar.

### 5.3.4 Butter characteristics

As indicated in Table 5.22, there were only small differences in butter characteristics between the two types of butter. The average iodine value of butter from "Protec"-fed herds was very slightly higher than that when the control diet was fed; this difference was not statistically significant ($p>0.05$, Appendix I).

The hardness of the butters complemented the level of unsaturation, and although the average hardness of "Protec"-butters was less than that of control butters, the difference was not significant ($p>0.05$). A similar pattern was noted for softening point, where the average SP of the control butter was 33.4°C and that of "Protec"-butters 32.2°C. Average oiling-off data obtained for control butters were not significantly different from those for "Protec"-butters ($p>0.05$). The difference noted between the two control butters was small and consistent with similar fluctuations in the iodine value and hardness. This trend

Table 5.21 Experiment III: Comparison of butter flavour using Signal Detection

| | R[1] |
|---|---|
| Control vs "Protec"-fed herds | 0.71 |

[1]Probability of distinguishing between flavour of two butter samples. Possible differences in spreadability were eliminated by whipping for 5 min in a Kitchen-Aid mixer.

Table 5.22 Effect of "Protec" on the quality of butter from commercial dairy herds

| | $C_1$ | $C_2$ | $C_{\bar{x}}$ | $P_1$ | $P_2$ | $P_{\bar{x}}$ | Dairy |
|---|---|---|---|---|---|---|---|
| Susceptibility to oxidation | | | | | | | |
| Peroxide value (meq/kg) | | | | | | | |
| - initial | 0.007 | 0.007 | 0.007 | 0.007 | 0.007 | 0.007 | 0.007 |
| - after one month storage | 0.068 | 0.024 | 0.046 | 0.061 | 0.023 | 0.042 | 0.062 |
| Characteristic | | | | | | | |
| Iodine value | 30.0 | 27.6 | 28.8 | 30.2 | 28.8 | 29.5 | 30.2 |
| Hardness ($kg/cm^2$) | 2.96 | 3.36 | 3.16 | 2.23 | 3.22 | 2.72 | 2.70 |
| Softening point ($^oC$) | 32.6 | 34.2 | 33.4 | 31.8 | 32.6 | 32.2 | 33.4 |
| Oiling-off (w/w %) | 1.91 | 1.37 | 1.64 | 1.99 | 1.63 | 1.81 | 1.88 |

[1]Differences between $C_{\bar{x}}$ and $P_{\bar{x}}$ were not significant ($P > 0.05$)

[2]Peroxide value of 0.50 meq/kg is considered to be of acceptable quality (Buchanan and Rogers, 1973).

was also apparent with butters resulting from the "Protec" diet.

### 5.3.5 Discussion

Data from this experiment were consistent with that reported in Experiments I & II, although trends were less obvious. The inclusion of "Protec" on a long term basis apparently had no effect on milk composition. Variability occurring in butter composition probably resulted during processing of the butters concerned. As indicated in sensory panels, milk and butter were of acceptable quality and free of off-flavours. This was true for butters even after prolonged refrigerated storage, implying that the long term use of "Protec" had no deleterious effects on the oxidative stability of butters produced. Properties of experimental butters such as unsaturation, hardness, and oiling were not drastically affected by the incorporation of "Protec" in the diets.

The lack of more pronounced responses may have been due to the grain and roughage intakes, lactation period of herds used, and the past history of the herds (i.e. high or low producers). Small differences that occurred in milk and butter characteristics on the same treatment are possibly due to slightly modified feeding regimes at the farms involved, and average lactation periods of the herds used.

According to unconfirmed reports by the involved farmers, no deleterious effects were noted when "Protec"-supplemented diets were fed, and the animals appeared more healthy. Thus, it may be concluded that the low-level use of PLFS in diets of lactating cows should not cause any undesirable quality changes in the dairy products obtained.

# 6. CONCLUSIONS AND RECOMMENDATIONS

## 6.1 Effect of "Protec" on milk quality

The results of this work were in agreement with other studies in which different vegetable oils such as safflower, sunflower, and linseed were used at higher levels of supplementation.

The inclusion of "Protec" in the diets of dairy cows at the 6% level of grain portion appears to be a possible "low-level optimum". At this level, milks were slightly less susceptible to hydrolytic rancidity and butters became significantly softer, without any apparent defects in milks and butters even after storage. Feeding oxidized "Protec" had no deleterious consequences in milk and butter, although the butter-softening effect that occurs with fresh "Protec" was less pronounced. Thus low levels of "Protec" can be fed to lactating dairy cows with no adverse effects on milk and butter quality even when fed on a long-term basis. The improved spreadability of butters and the lowered proness of milks to rancidity, are two important consequences that could encourage the continued use of PLFS.

## 6.2 Recommendations for future research

Variability in results may occur due to factors such as individual differences between cows, lactation period, and previous feeding history of cows. Consequently, interpretation of data becomes relatively difficult. The standardization of variables whenever possible should therefore be attempted in order for sound conclusions to be made. Constraints on the number and lactation stage of cows were among limitations that existed in this study due to its collaborative nature; this should be minimized in future research.

Difficulties experienced in assessing the level of protection of "Protec" (Jelen *et al.*, 1982) indicate that more work is needed in this area. This would enable more detailed understanding of the transfer of protected fatty acids to the milk fat. Such a method need to be fairly simple and quick so that application in a quality control situation can be made possible. The extent of "protection" in unprotected canola should also be investigated and correlated with the fatty acid profile of milkfat when this (unprotected canola) is fed.

Also, the effect of feeding protected canola on the trans fatty acids in milkfat should be investigated. It has been reported that the levels of these fatty acids are increased when unprotected canola is fed (Kennelly and Fenton, 1982); the trans fatty acids are considered to be undesirable components of processed vegetable fats.

The rumen bypass concept has been successfully applied in the area of dairy technology, and continued research in this area could provide more detailed understanding of the mechanism of protected fatty acid transfer, and the fate of protected protein.

## 7. REFERENCES


Allen, C. and Parks, O.W. 1975. Evidence for methional in skim milk exposed to sunlight. J. Dairy Sc. 58:1609.

Alsafar, T. 1974. Butterine made from rapeseed oil. Can. Inst. Food Sci. Technol. J. 7:220.

Amer, M.A. and Myhr, A.N. 1973. Modification of butter to improve temperature spreadability. Can. Inst. Food Sci. Technol. J. 6:261.

AOAC. 1980. Official Methods of Analysis of the Association of Official Analytical Chemists. Thirteenth Edition. AOAC, Washington, D.C.

AOCS. 1980. Official and Tentative Methods of the American Oil Chemists' Society. Vol.1, Third Edition, AOCS, Champaign, Ill.

Armstrong, D.G. and Ross, I.P. 1968. Principles of fat utilization. Proc. 2nd Nutr. Conf. Feed Manuf. Univ. Park Nottingham. Ed. Swan, H. and Lewis, D. Publ. J & A Churchill Ltd.

Astrup, H.N., Baevre, L., Vik-Mo, L. and Ekern, A. 1980. Effect on milk lipolysis of restricted feeding with and without supplementation with protected rape seed oil. J. Dairy Res. 47:287.

Astrup, H.N., Vik-Mo, L., Linstad, P. and Ekern, A. 1979. Casein protected oil supplement fed at a low level to milk cows. Milchwissenschaft 34:290.

Astrup, H.N., Vik-Mo, L., Ekern,A. and Baake, F.J. 1976. Feeding protected and unprotected oils to dairy cows. J. Dairy Sci. 59:426

Aurand, L.W., Boone, N.H. and Giddings, G.G. 1977. Superoxide and singlet oxygen in milk lipid peroxidation. J. Dairy Sci. 60:363

Aurand, L.W., Singleton, J.A. and Matrone, G. 1964. Sunlight flavour in milk. II. Complex formation between milk proteins and riboflavin. J. Dairy Sci. 47:827

Barbano, D.M. and Sherbon, J.W. 1980. Polyunsaturated protected lipid: effect on triglyceride molecular weight distribution. J. Dairy Sci. 63:731.

Barnard, S.E. 1973. Importance of shelf life for consumers of milk. J. Dairy Sci. 55:134.

Bergman, T., Berglof, and Kjell, S. 1962. Spontaneous oxidized (cardboard) flavour in raw milk and its influence on the quality of dairy products. Proc. XVI Int. Dairy Congr. Proc., Vol. A. Sect. II:1, 675, Copenhagen.

Bines, J.A., Brumby, P.E., Storry, J.E., Fulford, R.J. and Braithwaite, G.D. 1978. The effect of protected lipids on nutrient intakes, blood and rumen metabolites and milk secretion in dairy cows during early lactation. J. Agric. Sci. Camb. 91:135.

Bitman, J., Dryden, L.P., Goering, H.K., Wrenn, T.R., Yoncoskie, R.A. and Edmondson, L.F. 1973. Efficiency of transfer of polyunsaturated fats into milk. J. Am. Oil

Chem. Soc. 50:93.

Blaxter, K.L. 1967. The energy metabolism of ruminants. 2nd Ed. London, Hutchinson.

Blaxter, K.L. 1962. The energy metabolism of ruminants. Charles C. Thomas, Publ., Springfield, Il.

Brown, R.E. 1969. Conversion of nutrients into milk. In "Proc. 3rd Nutritional Conference for Feed Manufacture.

Bruhn, J.C., Franke, A.A. and Goble, G.S. 1976. Factors relating to development of sponaneous oxidized flavor in raw milk. J. Dairy Sci. 59:829.

Brumby, P.E., Storry, J.E., Bines, J.A. and Fulford, R.J. 1978. Utilization of energy for maintenance and production in dairy cows given protected tallow during early lactation. J. Agric. Sci. Camb. 91:151.

Brunner, J.R. 1980. Physical equilibrium in milk : the lipid phase. In "Fundamentals of Dairy Chemistry". Ed. Webb, B.H., Johnson, A.H. and Alford, J.A. AVI Publishing Co. Inc. Westport, Connecticut.

Buchanan, R.A. and Rogers, W.P. 1973. Manufacture of butter high in linoleic acid. Aus. J. Dairy Technol. 28:175.

Buchanan, R.A., Lawrence, A.J. and Loftus Hills, G. 1970. The properties of butter made from polyunsaturated milkfat. Proc. XVIIIth Int. Dairy Congr. 1E:511.

Carpenter, K.T. 1968. Possible adverse effects of oxidized fat in feeds. In "Proc. of 2nd Nutrional Conference for Feed Manufacturers. Ed. Swan, H. and Lewis, D.

Chandan, R.C. and Shahani, K.M. 1964. Milk lipases. A review. J. Dairy Sci. 47:471.

Chandler, N.J., Robinson, I.B., Ripper, I.C. and Fowler, P. 1973. The effect of feeding formaldehyde treated sunflower seed supplement on the yield and composition of milkfat. Aus. J. Dairy Technol. 29:179.

Christie, W.W. 1979. The effects of diet and other factors on the composition of ruminant tissues and milk. Prog. Lipid Res. 17:245.

Cook, L.J., Scott, T.W. and Pan, Y.S. 1972. Formaldehyde-treated casein-safflower oil supplement for dairy cows. II. Effect on the fatty-acid composition of plasma and milk lipids. J. Dairy Res. 39:211.

Cook, L.J. and Scott, T.W. 1970. Effects of feeding formaldehyde-treated lipid-protein particles to milking ruminants. Proc. XVIIIth Int. Dairy Congr. 1E:510.

Deeth, H.C. and Fitz-Gerald, C.H. 1977. Some factors involved in milk lipase activation by agitation. J. Dairy Res. 44:569.

Deeth, H.C. and Fitz-Gerald, C.H. 1976. Lypolysis in dairy products : A Review. Aus. J. Dairy Technol. 31:53

Dimick, P.S. 1973. Effect of fluorescent light on the flavour and selected nutrients of homogenized milk held in conventional containers. J. Milk Food Technol. 36:383.

Downey, W.K. 1980. Review of the progress of Dairy Science: Flavour impairment from pre- and post- manufacture lipolysis in milk and dairy products. J. Dairy Res. 47:237.

Downey, W.K. 1974. Enzyme systems influencing processing and storage of milk and milk products. Proc. XIXth Int. Dairy Congr. Vol.II. B.5(b):323.

Downey, W.K. and Murphy, R.F. 1975. Ann. Bull. Int. Dairy Fed. (Doc. 86):19. Cited in J. Dairy Res. 47:237 (1980).

Driessen, F.M. and Stadhouders, J. 1974. A study of spontaneous rancidity . Neth. Milk Dairy J. 28:130.

Dunkley, W.L. and Franke, A.A. 1967. Evaluating susceptibility of milk to oxidised flavour. J. Dairy Sci. 50:1.

Dunkley, W.L., Franklin, J.D. and Pangborn, R.M. 1962. Effects of fluorescent light on flavor, ascorbic acid and riboflavin in milk. Food Technol. 16(9):112.

Edmondson, L.F., Yoncoskie, R.A., Rainey, N.H., Douglas, F.W. Jnr. and Bitman, J. 1974. Feeding encapsulated oils to increase the polyunsaturation in milk and milkfat. J. Am. Oil Chem. Soc. 51:72.

Ensminger, M.E. and Olentine, G.G. Jr. 1978. Feeding Dairy Cattle. In "Feeds and Nutrition - Complete". First Ed. The Ensminger Publishing Co., Calif., U.S.A.

Fisher, L.J. 1975. Effect of pelleting on "Protec". Unpublished data. Canadian Dept. of Agriculture, Agassiz.

Fitz-Gerald, C.H. 1974. Milk lipase activation by agitation influence of temperature.

Fox, K.K., Holsinger, V.H., Caha, J. and Pallansch, M.J. 1960. Formation of a fat protein complex in milk by homogenization. J. Dairy Sci. 43:1396.

Franke, A.A., Dunkley, W.L. and Smith, N.E. 1977. Influence of feeding protected tallow on accuracy of analyses by Infrared Milk Analyzer. J. Dairy Sci. 60:1870.

Frankel, E.N., Nowakowska, J. and Evans, C.D. 1961. Formation of methyl azelaadehydate on autoxidation of lipids. J. Am. Oil Chem. Soc. 38:161

Goering, H.K., Wrenn, T.R., Edmondson, L.F., Weyant, J.R., Wood, D.L. and Bitman, J. 1977. Feeding polyunsaturated vegetable oils to lactating cows. J. Dairy Sci. 60:739. R.L. and Douglas, F.W.Jnr. 1976. Effect of feeding protected safflower oil on yield, composition, flavour and oxidative stability of milk. J. Dairy Sci. 59:416.

Goering, H.K., Gordon, C.H., Dryden, L.P., Wrenn, T.R., Bitman, J., Douglas, F.W. Jr. and Edmondson, L.F. 1972. Production effects of continuous feeding of protected safflower oil to milking cows. J. Dairy Sci. 55:714.

Goering, H.K., Gordon, C.H., and Wrenn, T.R., Bitman, J., King,

Gooden, J.M. and Lascelles, A.K. 1973. Effect of feeding protected lipid on the uptake of precursors of milkfat by the bovine mammary gland. Aus. J. Biol. Sci. 23:1201.

Gray, J.I. 1978. Measurement of lipid oxidation. J. Am. Oil Chem. Soc. 55:539.

Greenbank, G.R. 1940. Variation in the oxidation-reduction potential as a cause for the oxidized flavor in milk. J. Dairy Sci. 23:725.

Gregory, M.E., Hansen, A.P. and Aurand, L.W. 1972. Controlling light-activated flavour in milk. Am, Dairy Rev. 35:10.

Grieve, C.M. 1980. Protected fat in rations for lactating dairy cows. 59th Ann. Feeders Day Report, Fac. Agric./For., Univ. of Alberta, Edmonton, T6G 2N2.

Grieve, C.M. 1976. Protected fat in rations for lactating dairy cows. 55th Ann. Feeders Day Report, Fac. Agric./For., Univ. of Alberta, Edmonton, T6G 2N2.

Haase, G. 1977. Present status in research, development and commercialization of polyunsaturated meat and dairy products. 1. Biomanipulation of ruminant fats. Milchwissenschaft 32:257.

Hansen, A.P., Turner, L.G. and Aurand, L.W. 1975. Fluorescent light-activated flavour in milk. J. Milk Food Technol. 38:392.

Hedrick,T.I. and Glass, L. 1975. Chemical changes in milk during exposure to fluorescent light. J. Milk Food Technol. 38:129.

Hutton, J.B. 1974. Recent researches on feeding for milk production. Proc. XIXth Int. Dairy Congr. Vol. II:280.

Jack, E.L. and Smith, L.M. 1956. Chemistry of milk fat: A review. J. Dairy Sci. 39:1.

Jelen, P., Cadden, A.M., and Urquhart, A. 1982. Quality of milk products related to Protected Lipid Feed Supplements. Canadian Dairy Commission Project No. 03SU.01465-0-269.

Jenness, R. and Patton, S. 1959. Principles of Dairy Chemistry. John Wiley and Sons, Inc., New York.

Jensen, R.G. 1964. Symposium : Lipolysis. J. Dairy Sci. 47:210.

Johnson, A.H. 1980. The Composition of Milk. In "Fundamentals of Dairy Chemistry". Ed. Webb, B.H., Johnson, A.H. and Alford, J.A. AVI Publishing Co. Inc., Westport, Conn.

Johnson, A.R. 1974. Biomanipulation of ruminant fats. Implications for the food industry. Food Manuf. 49:19.

Josephson, D.V. and Doan, F.J. 1939. Observations on cooked flavor in milk - Its source and significance. The Milk Dealer 29:35.

Karel, M. 1973. Symposium : Protein interactions in biosystems. J. Food Sci. 38:756.

Kennelly, J.J and Fenton, M. 1982. Influence of feeding whole canola seed on the fatty acid composition of cows milk. 61st Annual Feeders' Day Report, Agric/For., Bull., Univ. of Alberta, Edmonton, T6G 2N2.

Kieseker, F.G. and Eustace, I.J. 1975. Manufacture by conventional churning of butter high in linoleic acid : Technology, physical properties and sensory evaluation. Aus. J. Dairy Technol. 37:17.

Kieseker, F.G., Hammond, L.A. and Zadow, J.G. 1974. Commercial scale manufacture of dairy products from milk containing high levels of linoleic acid. Aus. J. Dairy Technol. 36:51.

King, R.L. 1962. Oxidation of milk fat globule membrane material. 1. Thiobarbituric acid reaction as a measure of oxidized flavour in milk and model systems. J. Dairy Sci. 45:1165.

Kitchen, BJ. and Aston, J.W. 1970. Milk lipase activation. J. Dairy Technol. 32:10.

Koops, J. 1969. Effect of pH on the partition of natural and added copper in milk and cream. Neth. Milk Dairy J. 23:200.

Korycka-Dahl, M. and Richardson, T. 1980. Symposium: Initiation of oxidative changes in milk. Initiation of oxidative changes in foods. J. Dairy Sci. 63:1181.

Kreula, M. and Norlund, J. 1974. The manufacture of cultured butter from milk obtained by feeding cows with encapsulated oil. Proc. Int. Dairy Federation, New

Dehli. 133:108.

Kristensen, J.F., Andersen, P.E., Jensen, G.K., Hansen, P.S., Fisker, A.N. and Mortensen, B.K. 1974. Influence on milk yield fatty acid composition of milk fat and quality of milk and butter by feeding dairy cows encapsulated soybean oil. Denmark State Dairy and Livestock Husbandry. Report No.1.

Kronfeld, D.S. 1976. The potential importance of the proportions of glucogenic, lipogenic and aminogenic nutrients in regard to the health and productivity of dairy cows. Adv. Anim. Nutr. Anim. Physiol. 7:7.

Kronfeld, D.S., Donoghue, S., Naylor, J.M., Johnson, K. and Bradley, C.A. 1980. Metabolic effects of feeding protected tallow to dairy cows. J. Dairy Sci. 63:

Larmond, E. 1977. Laboratory Methods for Sensory Evaluation of Food. Canada Dept. of Agric. Publication #1637.

Luick, J.R. 1961. Synthesis of milkfat in the bovine mammary gland. J. Dairy Sci. 44:652.

Mahoney, M., Kulp, J. and Wheeler, L. 1979. Sensory detection of off-flavours in milk incorporating short-cut signal detection measures. J. Dairy Sci. 62:1857.

Marth, E.H., Ed. 1978. Standard Methods for the Examination of Dairy Products. 14th Edition. American Public Health Association.

Mattos, W. and Palmquist, D.L. 1974. Increased polyunsaturated fatty acid yields in milk of cows fed protected fat. J. Dairy Sci. 57:1050

McDonald, I.W. and Scott, T.W. 1977. Foods of ruminant origin with elevated content of polyunsaturated fatty acids. Unpublished. (Commonwealth Scientific and Industrial Research Organization).

McLeod, G.K., Yu, T. and Schaeffer, R. 1977. Feeding value of protected animal tallow for high yielding dairy cows. J. Dairy Sci. 60:726.

Mertens, W.G. and DeMan, J.M. 1972a. Automatic melting point determination of fats. J. Am. oil Chem. Soc. 49:366.

Mertens, W.G. and DeMan, J.M. 1072b. The influence of temperature treatment on solid-liquid ratios of fats determined by wide-line NMR. Can. Inst. Food Sci. Technol. J. 5:77.

Mills, S.C., Cook, L.J., Scott, T.W. and Nestel, P.J. 1976. Effect of dietary fat supplementation on the composition and positional distribution of fatty acids in ruminant and porcine glycerides. Lipids 11:49.

Mills, S.C., Sharry, L.F., Cook, L.K. and Scott, T.W. 1972. Metabolism of [14C] Formaldehyde when fed to ruminants as an aldehyde-casein-oil complex. Aus. J. biol. Sci. 25:802.

Mortensen, B.K. and Danmark, H. 1980. Method for the evaluation of the oiling-off tendency of butter. Milchwissenschaft 35:360.

Mulder, H. and Walstra, P. 1974. The Milk Fat Globule : Emulsion Science as applied to milk products and comparable foods. Farnham Royal, Bucks:Commonwealth Agricultural Bureaux.

Palmquist, D.L. and Jenkins, T.C. 1980. Fat in Lactation rations: A review. J. Dairy Sci. 63:1.

Palmquist, D.L. and Mattos, W. 1978. Turnover of lipoproteins and transfer to milk fat of dietary (1-Carbon-14) linoleic acid in lactating cows. J. Dairy Sci. 61:561.

Pan, Y.S., Cook, L.J. and Scott, T.W. 1972. Formaldehyde -treated casein-safflower oil supplement for dairy cows. 1. Effect on milk composition. J. Dairy Res. 39:203.

Parks, O. 1980. The lipids of milk: Deterioration PartII. Autoxidation. In "Fundamentals of Dairy Chemistry". Ed. Webb, B.H. Johnson, A.H. and Alford, J.A. AVI Publishing Co. Inc. Westport, Connecticut.

Parodi, P.W. and Dunstan, R.J. 1971. The relationship between the fatty acid composition, softening point and the Refractive Index of milkfat. Aus. J. Dairy Technol. 26:29.

Parr, A. 1982. Personal communication. Protec Division, Leduc Alfafa products. Leduc, Alberta.

Parr, A. 1980. Protected fat and protein for dairy cows. Alta Lipids Canada Ltd. (Internal Report - unpublished).

Parry, R.M. Jnr., Chandan, R.C. and Shahani, K.M. 1966. Rapid and sensitive assay for milk lipase. J. Dairy Sci. 49:356.

Patton, S. 1962. Dairy Products. In "Symposium on Foods: Lipids and Their Oxidation". ed. Schultz, H.W., Day, E.A. and Sinnhuber, R.O. AVI Publishing Co. Inc., Westport, Conn.

Patton, S. and Jensen, R.G. 1976. Biomedical Aspects of Lactation. Pergamon Studies in the Life Sciences. Pergamon Press.

Pillay, V.T., Myhr, A.N. and Gray, J.I. 1980a. Lipolysis in milk. 1. Determination of free fatty acid and threshold value for lipolyzed flavour detection. J. Dairy Sci. 63:1213.

Pillay, V.T., Myhr, A.N., Gray, J.I. and Biggs, D.A. 1980b. Lipolysis in milk. II. Effect of milking systems. J. Dairy Sci. 63:1219.

Plowman, R.D., Bitman, J., Gordon, CH., Dryden, L.P., Goering, H.K., Wrenn, T.R.., Edmondson, L.F., Yoncoskie, R.A. and Douglas, F.W. Jnr. 1972. Milkfat with increased polyunsaturated fatty acids. J. Dairy Sci. 55:204.

Rook, J.A.F. 1977. Dietary manipulation of the composition and quality of milk and milk products. J. Soc. Dairy Technol. 30:169.

Rook, J.A.F. and Thomas, P.C. 1969. Significance of rumen activity. Proc. 3rd Nutritional Conference for Feed Manufacturers. Univ. Park Nottingham. Ed. Swan, H. and Lewis, D. Publ. J & A Churchill Ltd.

Roessler, E.B., Pangborn, R.M., Sidel, J.L. and Stone, H. 1978. Expanded statistical tables for estimating significance in paired-difference, duo-trio and triangle tests. J. Food Sci. 43:940.

Scott, T.W. 1975. Polyunsaturated meat and dairy products. Cereal Foods 20:73.

Scott, T.W. and Cook, L.J. 1973. In "The Coronary Heart Disease and Dietary Fat Controversy. Commonwealth Scientific and Industrial Research Organization.

Scott, T.W., Bready, P.J., Royal, A.J. and Cook, L.J. 1972. Oilseed supplements for the production of polyunsaturated ruminant milkfat. Search 3:170.

Scott, T.W., Cook, L.J. and Mills, S.C. 1971. Protection of dietary polyunsaturated fatty acids against microbial hydrogenation in ruminants. J. Am. Oil Chem. Soc. 48:358.

Scott, T.W., Cook, L.J., Ferguson, K.A., McDonald, I.W., Buchanan, R.A. and Loftus Hills, G. 1970. Production of polyunsaturated milk fat in domestic ruminants. Aus.J. Sci. 32:291.

Shahani, K.M. 1974. Recent advances in the chemistry and physics of milk products for standardizing of processing and manufacturing. Proc. XIX Int. Dairy

Congr. Vol. II.

Sharpe, P.F. 1941. Intern. Assoc. Milk Dealers Bull., 20:523. Cited in "Fundamentals of Dairy Chemistry". 1980. 2nd Ed. AVI Publishing Co. Inc., Westport, Conn.

Shipe, W.F. 1980. Analysis and Control of Milk Flavour. In "The Analysis and Control of Less Desirable Flavors in Foods and Beverages". Ed. Charalambous, G. Academic Press.

Shipe, W.F. 1964. Oxidations in the dark. J. Dairy Sci. 47:221.

Shipe, W.F., Bassette, R., Deane, D.D., Dunkley, W.L., Hammond, E.G., Harper, W.J., Kleyn, D.H., Moprgan, M.E., Nelson, J.H. and Scalan, R.A. 1978. Off-flavours of milk: nomenclature, standards and bibliography. J. Dairy Sci. 61:855.

Sidhu, F.S., Brown, M.A. and Johnson, A.R. 1973. A sensitive method for detecting auto-oxidation in milk containing high levels of linoleic acid. J. Dairy Sci. 56:635.

Singleton, J.A., Aurand, L.W. and Lancaster, F.W. 1963. Sunlight flavor in milk. 1. A study of components involved in the flavor development. J. Dairy Sci. 46:1050.

Sinnhuber, R.O., Yu, T.C. and Yu, Te Chang. 1958. Characterization of the red pigment formed in the 2-TBA determination of oxidative rancidity. Food Res. 23:626.

Smith, G.J. and Dunkley, W.L. 1962. Pro-oxidants in spontaneous development of oxidised flavor in milk. J. Dairy Sci. 45:170.

Smith, N.E., Dunkley, W.L. and Franke, A.A. 1978. Effects of feeding protected tallow to dairy cows in early lactation. J. Dairy Sci. 61:747.

Smith, N.E., Dunkley, W.L. and Ronning, M. 1963. Influence of linoleic acid content of milk lipids on oxidation of milk and milkfat. J. Dairy Sci. 46:7.

Sporns,P 1982. Unpublished data.

Stanley, D.W., Gill, T.A., deMan, J.M. and Tung, M.A. 1976. Microstructure of rapeseed. Can. Inst. Food Sci. Technol. J. 9:54.

Storry, J.E. 1981. The Effect of Dietary Fat on Milk Composition. In "Recent Advances in Animal Nutrition - 1981. Ed. Haresign, W. Studies in the Agricultural and Food Sciences. Butterworths.

Storry, J.E. 1972. Milk fat: Its synthesis and composition in relation to the nutrition of the dairy cow. J. Soc. Dairy Technol. 25:40.

Storry, J.E. 1970. Reviews of the progress of Dairy Science. Section A. Physiology. Ruminant metabolism in relation to the synthesis and secretion of milk fat. J. Dairy Res. 37:139.

Storry, J.E. and Brumby, P. 1979. Protected lipids for dairy cows. Big Farm Management 9(2):64.

Storry, J.E., Brumby, P.E., Bines, J.A., Fulford, R.J. and Braithwaite, G.D. 1978a. Milk composition and digestibility of nutrients in dairy cows fed varying levels of protected tallow. Proc. XXth Int. Dairy Congr. p.65.

Storry, J.E., Brumby, P.E. and Tuckley, B. 1978b. Effects of feeding protected tallow to dairy cows in early lactation on the yield and composition of milk fat. Proc. XXth Int. Dairy Congr. p. 64.

Storry, J.E., Brumby, P.E. and Cheeseman, G.C. 1974. Protected lipids in ruminant nutrition. ADAS. Quart. Rev. 15:96.

Tamsma, A., Mucha, T.J. and Pallansch, M.T. 1962. Factors related to flavor stability of foam-dried milk. II. Effect of heating milk prior to drying. J. Dairy Sci. 45:1435.

Tarassuk, N.P. and Frankel, E.N. 1955. The mechanism of activation of lipolysis and the stability of lipase systems in normal milk. J. Dairy Sci. 38:438.

Taylor, M.W. and Norris, R. 1977. The physical properties of dairy spreads. N.Z. J. Dairy Sci. Technol. 12:166 The Food and Drugs Act. (Canada). As ammended to January 14, 1982. Section B.08. 056.

Thomas, E.L., Nielsen, A.J. and Olson, J.C. Jnr. 1955. Hydrolytic rancidity in milk. A simplified method for estimating the extent of its development. Am. Milk Rev. 17:50

Wong, W., Jelen,P. and DeMan, J.B. 1982. Softening of butter related to feeding low doses of protected tallow feed supplement. J. Dairy Sci. 65:1632.

Wood, F.W., Murphy, M.F. and Dunkley, W.L. 1975. Influence of elevated polyunsaturated fatty acids on processing and physical properties of butter. J. Dairy Sci. 58:839.

Wrenn, T.R., Bitman, J., Waterman, R.A., Weyant, J.R., Wood, D.L., Stronzinski, L.L. and Hooven, N.W. Jnr. 1978. Feeding protected and unprotected tallow to lactating cows. J. Dairy Sci. 61:49.

Yang, Y.T., Rhode, J.M. and Baldwin, R.L. 1978. Dietary lipid metabolism in lactating dairy cows. J. Dairy Sci. 61:1400.

*APPENDIX A* Calving Dates of Cows Used in Experiments I & II

| Cow I.D. No. | Pair | Calving Date |
|---|---|---|
| Experiment I | | |
| 812 | 1 | Aug. 8/81 |
| 610 | 1 | June 24/81 |
| 832 | 2 | July 4/81 |
| 613 | 2 | June 19/81 |
| 833 | 3 | July 25/81 |
| 824 | 3 | July 9/81 |
| 834 | 4 | July 23/81 |
| 841 | 4 | Sept. 2/81 |
| Experiment II | | |
| 708 | -[1] | May 31/81 |
| 613 | -[1] | June 19/81 |
| 824 | -[1] | July 9/81 |
| 814 | -[1] | April 28/81 |
| 719 | -[2] | May 23/81 |
| 855 | -[2] | Sept. 30/81 |
| 601 | -[2] | May 6/81 |
| 632 | -[2] | Aug. 19/81 |
| 610 | -[3] | June 24/81 |
| 841 | -[3] | Sept. 2/81 |
| 453 | -[3] | May 10/81 |
| 306 | -[3] | May 15/81 |

[1]Diet 1 (0% 'Protec')

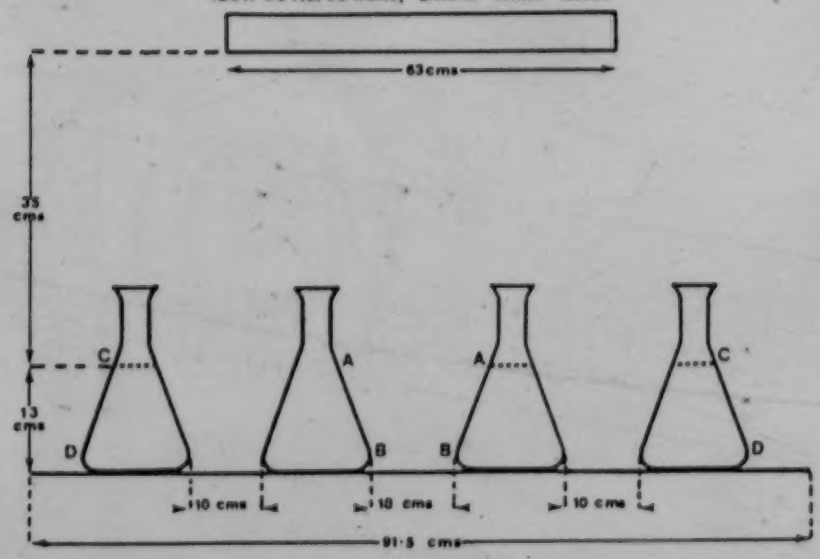
[2]Diet 2 (6% 'Fresh Protec')

[3]Diet 3 (6% 'Stored Protec')

*Experiment I was conducted from Oct. 15/81 to Jan. 15/82, while Experiment II was conducted from March 2/82 to March 23/82.

APPENDIX B ------ Fluorescent Light Irradiation of Milk.

G. E. "Bright-Stik" Fluorescent Light Fixture.
120v. 60 Hz. 33 watts, Deluxe White Bulb.




Light Intensity at Points (Measured by Gussen Paniux Lightmeter)

| | | | |
|---|---|---|---|
| A | 50 ft. candles | = 538° lux | |
| B | 33 " " | | |
| C | 28 " " | = 301° lux | |
| D | 21 " " | | |

APPENDIX C ----- *QUESTIONNAIRE FOR TRIANGLE TEST*

*Name:* _____ *Date:* _____

*Test:* _____

Two of these three samples are identical, the third is different.

1. Taste the samples in the order indicated and identify the odd sample.

| Code | Check Odd Sample |
|---|---|
| _____ | |
| _____ | |
| _____ | |

2. Indicate the degree of difference between the duplicate samples and the odd sample.

Slight _____

Moderate _____

Much _____

Extreme _____

3. Describe the type of difference between the duplicate and the odd sample.

APPENDIX D. Instructions and questionnaire for Triangle Test

## INSTRUCTIONS

You have been given three sets of butter samples. Please evaluate each set independently for differences in spreadability and taste.

### Spreadability

Spread a small amount of butter on a cracker and compare the spreadability of the three test butters. Wipe the knife clean between samples.

### Taste

Taste each butter. Use of the supplied crackers is optional, but please be consistent.

Please specify in question 3 if the differences detected among samples were differences in spreadability and/or taste.

APPENDIX D cont'd.

QUESTIONNAIRE FOR TRIANGLE TEST

Name: _____ Date: _____

Test: _____

Two of these three samples are identical, the third is different.

1. Compare the samples in order indicated and identify the odd sample.

| Code | Check Odd Sample |
|---|---|
| _____ | |
| _____ | |
| _____ | |

2. Indicate the degree of difference between the duplicate samples and the odd sample.

Slight _____

Moderate _____

Much _____

Extreme _____

3. Describe the type of difference between the duplicate and the odd sample. (Spreadability = S; Taste = T; Both = B)

APPENDIX E Flavour Evaluation of butter using Signal Detection.

Experiment: _____ Date: _____

Name: _____

Compare each test sample with the reference sample marked "R". Determine if the test samples differ in flavour from the reference sample. Please taste the samples in the order presented. The reference sample "R" may be tasted as often as necessary.

| SAMPLE | DIFFERENT | NOT DIFFERENT | NOT SURE |
|---|---|---|---|
| | | | |
| | | | |
| | | | |
| | | | |
| | | | |

COMMENTS:

APPENDIX F Experiment I: Mean Squares of data obtained from Analysis of Variance and Orthogonal Contrasts of individual cows

| | Source of Variation | | | | |
|---|---|---|---|---|---|
| | Cow Pairs | Period | Diet | | Error |
| | | | Total | Linear | |
| Susceptibility of milks to induced hydrolytic and oxidative rancidity | | | | | |
| Acid Degree Value (0 min) | 0.081 (3) | 0.874 (3) | 0.048 (3) | 0.132 (1) | 0.332 (6) |
| (15 min) | 0.027 (3) | 0.672 (3) | 0.322 (3) | 0.619 (1) | 0.121 (30) |
| (30 min) | 0.807 (3) | 1.091* (3) | 1.484* (3) | 2.938* (1) | 0.220 (6) |
| (60 min) | 0.143 (3) | 2.655* (3) | 0.916 (3) | 2.072* (1) | 0.131 (3) |
| Thibarbituric Acid (initial) | 0.002 (3) | 0.276 (3) | 0.007 (3) | 0.010 (1) | 0.000 |
| (Cu) | 0.058 (3) | 2.666** (3) | 0.116 (3) | 0.003 (1) | 37.75 (6) |
| Fl. Light 3 days | 0.052 (3) | 0.129* (3) | 0.065 (3) | 0.000 (1) | 18.253 (4) |
| Fl. Light 5 days | 0.120 (3) | 0.060 (3) | 0.021 (3) | 0.004 (1) | 56.200 (5) |

Numbers in parentheses indicate degrees of freedom.

*P < 0.05, **P < 0.001

APPENDIX G Experiment I: Mean Squares of data obtained from Analysis of Variance and Orthogonal Contrasts of composite milk samples

| | Source of Variation | | | | |
|---|---|---|---|---|---|
| | Cow Pairs | Period | Diet | | Error |
| | | | Total | Linear | |
| **Butter Characteristics** | | | | | |
| Iodine Value | 36.729* (3) | 2.895 (3) | 22.562 (3) | 59.512 (1) | 6.396 (6) |
| Force | 0.767** (3) | 0.301* (3) | 0.767 (3) | 2.212** (1) | 0.056 (6) |
| Softening Point | 2.245 (3) | 1.285 (3) | 0.487 (3) | 1.057 (1) | 0.653 (6) |
| Dropping Point | 2.168* (3) | 0.764 (3) | 1.769* (3) | 2.550 (1) | 0.269 (4) |
| Oiling Off | 0.762 (3) | 0.007 (3) | 0.276 (3) | 0.181 (1) | 0.233 (5) |
| Solid Fat (0°C) | 50.802 (3) | 10.715 (3) | 31.461 (3) | 45.344 (1) | 19.461 (4) |
| (5°C) | 36.611 (3) | 11.987 (3) | 19.677 (3) | 33.610 (1) | 15.212 (4) |
| (10°C) | 35.193 (3) | 7.951 (3) | 21.218 (3) | 25.964 (1) | 14.250 (4) |
| (15°C) | 34.955 (3) | 11.146 (3) | 15.481 (3) | 7.467 (1) | 10.940 (4) |
| (20°C) | 36.500 (3) | 15.364 (3) | 17.843 (3) | 7.027 (1) | 12.722 (4) |
| (25°C) | 6.587* (3) | 3.432* (3) | 3.194* (3) | 4.946* (1) | 0.216 (3) |

Numbers in parentheses indicate degree of freedom.

*$p < 0.05$, **$p < 0.001$

*APPENDIX H* Experiment II: Mean Squares of Data Obtained from Analysis of Variance

| | Source of Variation[1] | |
|---|---|---|
| | Between Groups[2] | Within Groups[3] |
| Feed Consumption | | |
| Hay | 1.211 | 0.776 |
| Concentrate | 0.351 | 0.866 |
| Milk Yield & Composition | | |
| Milk yield | 1.764 | 6.877 |
| Butterfat (IR) | 0.071 | 0.530 |
| Butterfat (Moj) | 0.084 | 0.526 |
| FCM (IR) | 1.716 | 10.788 |
| FCM (Moj) | 1.525 | 10.868 |
| ($Butterfat_{IR}$-$Butterfat_{Moj}$) | 0.002 | 0.004 |
| Protein | 0.078 | 0.022 |
| Lactose | 0.003 | 0.019 |
| Total solids | 0.185 | 0.416 |
| Solids-not-fat | 0.136 | 0.066 |
| Butterfat and Protein Yield (g) | | |
| Butterfat (IR) | 0.004 | 0.034 |
| Butterfat (Moj) | 0.004 | 0.034 |
| Protein | 0.001 | 0.007 |

[1] 11df

[2] 2df

[3] 9df

*APPENDIX* I Experiment III: Mean Squares of Data Obtained from Analysis of Variance

| | Source of Variation[1] | |
|---|---|---|
| | Between Groups[2] | Within Groups[3] |
| Milk Composition | | |
| Butterfat (IR) | 0.004 | 0.007 |
| Butterfat (Moj) | 0.010 | 0.007 |
| ($Butterfat_{IR}$ - $Butterfat_{Moj}$) | 0.001* | 0.000 |
| Protein | 0.001 | 0.008 |
| Lactose | 0.005 | 0.001 |
| Total solids | 0.053 | 0.010 |
| Solids non fat | 0.027 | 0.007 |
| Butter Characteristics | | |
| Iodine value | 0.250 | 1.25 |
| Force | 0.189 | 0.285 |
| Softening point | 1.440 | 0.800 |
| Oiling-off | 0.029 | 0.105 |
| Moisture | 0.298 | 0.345 |

[1] 3 df

[2] 1 df

[3] 2 df